

PARENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year)
16 août 2001 (16.08.01)

From the INTERNATIONAL BUREAU

To:

IRVINE, Claire, Jonquil
J.A. Kemp & Co.
14 South Square
Gray's Inn
London WC1R 5LX
ROYAUME-UNI

Date of mailing (day/month/year) 16 août 2001 (16.08.01)	International filing date (day/month/year) 07 juillet 2000 (07.07.00)		
Applicant's or agent's file reference N.75756D JCI	IMPORTANT NOTIFICATION		
International application No. PCT/GB00/02623			

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address WEAVER, Donald 27 College View Crescent Kingston, Ontario K7M 7J8 Canada	State of Nationality CA	State of Residence CA
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
Deleted from record.

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer R. Raissi
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PARENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year)
08 February 2001 (08.02.01)

From the INTERNATIONAL BUREAU

To:

IRVINE, Claire, Jonquil
J.A. Kemp & Co.
14 South Square
Gray's Inn
London WC1R 5LX
ROYAUME-UNI

Applicant's or agent's file reference
N.75756D JCI

IMPORTANT NOTIFICATION

International application No.
PCT/GB00/02623

International filing date (day/month/year)
07 July 2000 (07.07.00)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address

QUEENS UNIVERSITY AT KINGSTON
Kingston
Ontario K7L 3N6
Canada

State of Nationality

State of Residence

CA

CA

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

Please note additional applicant for all designated States except US.

4. A copy of this notification has been sent to:

the receiving Office

the designated Offices concerned

the International Searching Authority

the elected Offices concerned

the International Preliminary Examining Authority

other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Jean-Marie McAdams

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Date of mailing (day/month/year)

22 March 2001 (22.03.01)

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

International application No.

PCT/GB00/02623

Applicant's or agent's file reference

N.75756D JCI

International filing date (day/month/year)

07 July 2000 (07.07.00)

Priority date (day/month/year)

09 July 1999 (09.07.99)

Applicant

CLARK, Anne et al

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

09 February 2001 (09.02.01)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Olivia TEFY

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

10/030350

**RECOMMENDED
AANGEOTEKEN**

PCT

From the INTERNATIONAL SEARCHING AUTHORITY

To:
J.A. KEMP & CO.
 Attn. IRVINE, CLAIRE
 14 South Square
 Gray's Inn
 London WC1R 5LX
 UNITED KINGDOM

J.A. KEMP & CO.

Rec'd. 21 DEC 2001

Action by.....

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

Applicant's or agent's file reference
N.75756D JCI

International application No.
PCT/GB 00/ 02623

Date of mailing
 (day/month/year)

19/12/2001**PAYMENT DUE**

within **30** ~~XX~~ days from the above date of mailing

International filing date
 (day/month/year)

07/07/2000

Applicant

QUEENS UNIVERSITY AT KINGSTON

1. This International Searching Authority

(i) considers that there are **10** (number of) Inventions claimed in the international application covered by the claims indicated ~~below~~ on the extra sheet:

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~below~~ on the extra sheet:

(ii) has carried out a partial international search (see Annex) will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:
1-3, 8, 10-12, 16-18, 21, 52 partially

(iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby invited, within the time limit indicated above, to pay the amount indicated below:

GBP 624,00 x **9** = **GBP 5,616,00**
 Fee per additional invention number of additional inventions total amount of additional fees

Or. **FUR 945,00** x **9** = **FUR 8,505,00**

The applicant is informed that, according to Rule 40.2(c), the payment of any additional fee may be made under protest, i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

3. Claim(s) Nos. **See annex** Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the International Searching Authority

European Patent Office, P.O. 5818 Patentlaan 2
 NL-2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 840-3016

Authorized officer

Joannes Vergoosen

International application No.

PCT/GB 00/02623

INVITATION TO PAY ADDITIONAL FEES

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid, 3-amino-2-hydroxy-1-propanesulfonic acid, and 3-dimethylamino-1-propanesulfonic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

2. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of DL-2-amino-5-phosphovaleric acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

3. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 4-Phenyl-1-(3'-sulforpropyl)-1,2,3,6-tetrahydropyridine for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

4. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of cyclohexylsulfamic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial

amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

5. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of O-phospho-L-serine for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

6. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of hexafluoroglutaric acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

7. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 8-methoxyquinoline-5-sulfonic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

8. Claims: 1,8,10,16,21 (partially) 4,5,13,14,19,20

Use of compounds of formula of claim 4, including 1,2,3,4-tetrahydroisoquinoline for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy,

INVITATION TO PAY ADDITIONAL FEES

International application No.

PCT/00/02623

primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

9. Claims: 6,9,15,22-44(1)

Process for the preparation of a cell according to claims 22-31, ex vivo cells produced by such process according to claims 35-41, and culture mediums according to claim 32-34, a vessel and kit according to claims 43-44.

10. Claims: 44(2),46-51

Use of an antibody that binds an inhibitor or compound as defined in claim 2,4 or 27, or of a fragment of said antibody that retains the ability to bind the said inhibitor or compound, to identify a substance that can be used to prepare cells for transplantation in a process according to claim 22 or 23 and a method of identifying an inhibitor that can be used to prepare cells for transplantation in a process according to claim 22 or 23, comprising contacting a candidate substance with a mammalian cell and determining whether the candidate substance inhibits the formation of fibrils or causes the breakdown of fibrils, (i) the inhibition of formation of fibrils or (ii) the breakdown of fibrils, indicating that the substance is an inhibitor that can be used in said process, and inhibitors identified by such method (sic).

The problems to be solved by the present application are
 (i) to provide for a medicament for inhibiting IAPP-associated amyloid deposits in a disorder where such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.
 (ii) to provide for cells suitable for transplantation into a mammal,
 (iii) to provide for a culture medium or a culture medium pre-mix,
 (iv) to provide for ex vivo cells,
 (v) to provide for an antibody that binds an inhibitor or compound as defined in claim 2,4 or 27, or of a fragment of said antibody that retains the ability to bind the said inhibitor or compound,
 (vi) to provide for a method of identifying an inhibitor that can be used to prepare cells for transplantation.

The proposed solutions for the problems (i)-(iv) is to use a compound of the formula of claim 2 or of the formula of claim 4, more particularly selected from the group consisting of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid;

4-Phenyl-1-(3'-sulforpropyl)-1,2,3,6-tetrahydropyridine, cyclohexylsulfamic acid, O-phospho-L-serine, hexafluoroglutaric acid, 8-methoxyquinoline-5-sulfonic acid, 3-amino-2hydroxy-1-propanesulfonic acid, 3-dimethylamino-1-propanesulfonic acid, and 1,2,3,4-tetrahydroisoquinoleine.

WO9637612 discloses the treatment of an animal having disease characterised by an over expression of an IAPP gene prod. comprising the admin. of an IAPP gene prod. over expression inhibitor, and methods for identifying compounds that inhibit IAPP gene prod. over expression, see the passages cited in the search report.

WO9422437 discloses the inhibition if amyloid deposition, treatment of Alzheimer's disease, Down's syndrome, hereditary cerebral haemorrhage amyloidosis (dutch); amyloid A (reactive (secondary/amyloidosis)) with e.g. 2-aminoethanesulfonic acid (taurine), a compound of the formula of claim 2 , see the passages cited in the search report.

WO9628187 discloses the use of taurine, 3-cyclohexylamino-1-propanesulfonic acid, and 2-aminoethylhydrogen sulfate (compounds of the formula of claim 2 to inhibit amyloid deposition in a subject see the passages cited in the search report.

SCHWARCZ R. ET AL in LANCET, (1985) 2/8447 (140-143) disclose the use of several aminoalkylphosphonates including 2-amino-5-phosphonovalerate (a compound of claim 3), as excitatory aminoacid antagonists useful in treating Alzheimer's, huntington's disease, see the passages cited in the search report.

WO9303714 discloses the use of 3-amino propanesulfonic acid, taurine and 4-amino-trans crotonic acids (compounds of the formula of claim 2) to treat Non-insulin dependent diabetes mellitus.

WO9811923 discloses methods for identifying agents for treating neurodegenerative disease or disorders, e.g. Alzheimer's disease, hereditary haemorrhage with amyloidosis-Dutch type, cerebral amyloid angiopathy, cerebral amyloid angiopathy, Down's syndrome, spongiform encephalopathy, Creutzfeld-Jakob disease etc., by using IAPP-associated amyloid deposition inhibition.

The documents cited do not represent a comprehensive search for any of the defined inventions and are to be considered only as part of the prior art pertaining to the general idea underlying the present application.

According to Article 3(4)(ii) PCT, an international application shall comply with "the prescribed requirement of unity of invention". This means, as explained in Rule 13.1 PCT, that the application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept.

From the above cited document, it appears that the idea to use aforementioned compounds for the specified therapeutic applications is known in the prior art and can not fulfil the role of special technical feature (general inventive concept) in the sense of rule 13.2 PCT.

International application No.

PCT/GB 00/02623

INVITATION TO PAY ADDITIONAL FEES

In the present application no further technical feature can be distinguished that can be regarded as a "special technical feature" involved in the technical relationship among the different inventions.

Consequently the present application lacks unity of invention.

Each of the invention is a distinct invention, characterised by its own special technical feature, defining the contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

However in order to limit the number of subject matters as much as possible, the subject matter was regrouped according to structural similarities.

As searching the other subject would have caused a major additional searching effort, only the first invention was searched.

The application has been divided into the above (groups of) inventions which individually are considered to meet the requirement of unity. If additional fees are paid for (one or more of) the, as yet, unsearched invention(s), the subsequent search(es) might reveal prior art which leads to a finding of lack of unity a posteriori within (one or more of) the, as yet, unsearched invention(s). Should this be the case, as a rule, no further invitation to pay additional fees will be issued. Only the first identified invention in each group of inventions, for which additional search fees have been paid in due time and which subsequently is considered to lack unity, will be searched.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 206

Continuation of Box 3.

Although claims 1-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Further defect(s) under Article 17(2)(a):

Continuation of Box 3.

Present claims 1-21,52. relate to a product/therapeutic application defined by reference to a desirable characteristic or property, namely inhibiting IAPP-associated amyloid deposits.

The claims cover all product/therapeutic application having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such product/therapeutic application. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/therapeutic application by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Present claims 2.11 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the product/therapeutic application relating to the treatment of diabetes (see claim 7) as far as related to the first invention.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

Annex to Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS
OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/GB 00/02623

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
see 'Invitation to pay additional fees.'
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.
3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.
4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 37612 A (PFIZER ;CARTY MAYNARD D (US); KREUTTER DAVID K (US); SOELLER WALTE) 28 November 1996 (1996-11-28) abstract page 2, line 16 -page 4, line 3 ---	1
X	WO 94 22437 A (UNIV KINGSTON) 13 October 1994 (1994-10-13) abstract page 1, line 33 -page 2, line 36 page 4, line 16-32 page 13, line 10-17 page 15, line 21 -page 16, line 2; claim 1 ---	1,2,8, 10,11, 17,21,52
X	WO 96 28187 A (KISILEVSKY ROBERT ;SZAREK WALTER (CA); UNIV KINGSTON (CA); WEAVER) 19 September 1996 (1996-09-19) abstract page 5, line 1 -page 6, line 5 page 12, paragraph 4 -page 14, paragraph 1 page 17, paragraph 1; table 2 ---	1,2,8, 10,11, 17,21,52
X	WO 94 27602 A (CORTEX PHARMA INC) 8 December 1994 (1994-12-08) abstract page 3, line 16 -page 4, line 15 page 4, line 21 -page 6, line 31 page 9, line 23 -page 10, line 25; claim 16; example 16 ---	1,2 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *D* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more others such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

COMMUNICATION RELATING TO THE RESULTS
OF THE PARTIAL INTERNATIONAL SEARCHInternational Application No
PCT/GB 00/02623

C.(Continuation) DOCUMENTS CONSIDERED RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"USE OF METABOTROPIC AGONISTS IN PROGRESSIVE NEURODEGENERATIVE DISEASES" EXPERT OPINION ON THERAPEUTIC PATENTS. ASHLEY PUBLICATIONS, GB, vol. 5, no. 5, 1995, pages 491-493, XP001012270 ISSN: 1354-3776 the whole document ---	1,2
X	SCHWARCZ R. ET AL: "Excitatory aminoacid antagonists provide a therapeutic approach to neurological disorders." LANCET, (1985) 2/8447 (140-143). CODEN: LANCAO, XP001030857 abstract page 141, column 2, paragraph 3 -page 143, column 1, paragraph 2 ---	1,2
X	WO 93 03714 A (UPJOHN CO) 4 March 1993 (1993-03-04) abstract page 4, line 33 -page 6, line 5 page 11, line 1-25; claims 1-9; tables 3,6,8 ---	1,2,7,8, 10,11, 16,17, 21,52
X	COPANI A ET AL: "ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS PROTECTS CULTURED NEURONS AGAINST APOPTOSIS INDUCED BY beta-AMYLOID PEPTIDE" MOLECULAR PHARMACOLOGY, BALTIMORE, MD, US, vol. 5, no. 47, 1995, pages 890-897, XP002079923 ISSN: 0026-895X abstract page 890, column 1-2; figure 3 page 893, column 2, paragraph 2 -page 894, column 1, paragraph 1; figure 5 page 895, column 2, paragraph 1 -page 896, column 1, paragraph 2 --- -/-	1,2

COMMUNICATION RELATING TO THE RESULTS
OF THE PARTIAL INTERNATIONAL SEARCHInternational Application No
PCT/GB 00/02623

C.(Continuation) DOCUMENTS CONSIDERED RELEVANT

Category *	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
X	HUTCHINGS R ET AL: "The effect of excitotoxin antagonists on ibotenic acid-induced alteration of APP mRNA hippocampal expression." JOURNAL OF PHARMACY AND PHARMACOLOGY, vol. 47, no. 12B, 1995, page 1131 XP002079922 British Pharmaceutical Conference 1995: Science Proceedings of the 132nd Meeting; Warwick, England, UK; September 15-18, 1995 ISSN: 0022-3573 abstract	1,2
X	PACHE D M ET AL: "EFFECT OF SELECTIVE EXCITATORY AMINO ACID ANTAGONISTS ON EXCITOTOXIN-INDUCED CHANGES IN APP mRNA EXPRESSION" PHARMACOLOGY REVIEWS AND COMMUNICATIONS, GORDON AND BREACH, CH, vol. 10, no. 3, 1999, pages 205-212, XP001014486 abstract	1,2
X	WO 98 11923 A (Baylor College Medicine ;GIULIAN DANA J (US)) 26 March 1998 (1998-03-26) the whole document	1
E	WO 00 71101 A (NEUROCHEM INC ;UNIV KINGSTON (CA)) 30 November 2000 (2000-11-30)	1-3,7,8, 10-12, 16-18, 21,52
E	the whole document	
WO 00 64420 A (KONG XIANQI ;NEUROCHEM INC (CA); SZAREK WALTER (CA); UNIV KINGSTON) 2 November 2000 (2000-11-02)	1-3,7,8, 10-12, 16-18, 21,52	
X,P	abstract page 2, line 15 -page 4, line 25 page 13, line 10 -page 15, line 13 page 22, line 21 -page 23, line 12; claims 1-3,9,12-16,20-22,25-65; examples 3-8	1-3,7,8, 10-12, 16-18, 21,52
WO 99 40909 A (NEUROCHEM INC) 19 August 1999 (1999-08-19)		
X,P	abstract page 3, line 3-14 page 12, line 7-19 claims 1-12	1-3,7,8, 10-12, 16-18, 21,52

Patent Family Annex

Information on patent family members

International Application No

PCT/GB 00/02623

Patent document cited in search report		Application date	Patent family member(s)		Publication date
WO 9637612	A	28-11-1996	US CA EP WO JP	6187991 B1 2219629 A1 0827540 A1 9637612 A1 10507084 T	13-02-2001 28-11-1996 11-03-1998 28-11-1996 14-07-1998
WO 9422437	A	13-10-1994	CA CA WO WO EP EP EP JP US US US US	2159326 A1 2159649 A1 9422437 A2 9422885 A1 1060750 A2 0691844 A1 0691976 A1 8508260 T 5643562 A 5972328 A 5728375 A 5840294 A	13-10-1994 13-10-1994 13-10-1994 13-10-1994 20-12-2000 17-01-1996 17-01-1996 03-09-1996 01-07-1997 26-10-1999 17-03-1998 24-11-1998
WO 9628187	A	19-09-1996	US US US AU AU BR CA WO EP JP US	5643562 A 5972328 A 5840294 A 716218 B2 5097696 A 9607197 A 2213759 A1 9628187 A1 0814842 A1 11501635 T 5728375 A	01-07-1997 26-10-1999 24-11-1998 24-02-2000 02-10-1996 11-11-1997 19-09-1996 19-09-1996 07-01-1998 09-02-1999 17-03-1998
WO 9427602	A	08-12-1994	AU WO US	6836394 A 9427602 A1 5622981 A	20-12-1994 08-12-1994 22-04-1997
WO 9303714	A	04-03-1993	AU AU AU AU CA EP JP WO	664710 B2 2407592 A 3061495 A 3061595 A 2113817 A1 0600973 A1 6510760 T 9303714 A2	30-11-1995 16-03-1993 09-11-1995 09-11-1995 04-02-1993 15-06-1994 01-12-1994 04-03-1993
WO 9811923	A	26-03-1998	US US AU AU EP WO US US	6071493 A 6043283 A 738509 B2 4589497 A 1051195 A1 9811923 A1 2001016326 A1 2001016327 A1	06-06-2000 28-03-2000 20-09-2001 14-04-1998 15-11-2000 26-03-1998 23-08-2001 23-08-2001
WO 0071101	A	30-11-2000	AU AU WO WO	4282400 A 4905000 A 0064420 A2 0071101 A2	10-11-2000 12-12-2000 02-11-2000 30-11-2000

Patent Family Annex

Information on patent family members

International Application No

PCT/GB 00/02623

Patent document cited in search report	Application date		Patent family member(s)		Publication date
WO 0064420	A 02-11-2000	AU	4282400 A		10-11-2000
		WO	0064420 A2		02-11-2000
		AU	4905000 A		12-12-2000
		WO	0071101 A2		30-11-2000
WO 9940909	A 19-08-1999	AU	2437899 A		30-08-1999
		EP	1054664 A1		29-11-2000
		WO	9940909 A1		19-08-1999

101030350

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference N. 75756D JCI	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 00/ 02623	International filing date (day/month/year) 07/07/2000	(Earliest) Priority Date (day/month/year) 09/07/1999
Applicant QUEENS UNIVERSITY AT KINGSTON		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of **11** sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
 - contained in the international application in written form.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority in written form.
 - furnished subsequently to this Authority in computer readable form.
 - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. **Certain claims were found unsearchable** (See Box I).

3. **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. **---**

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/GB 00/02623**Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)**

Methods and compositions are provided for inhibiting, preventing and treating amyloid depositions, e.g. in pancreatic islets, wherein the amyloidotic deposits are islet amyloid polypeptide (IAPP)-associated amyloid deposition or deposits. Accordingly, the compositions and method of the invention are useful for inhibiting diabetes. The invention also provides a process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming fibrils, said process comprising contacting the cells with an inhibitor of fibril formation. In particular the process prepares cells for use in a method of treating diabetes. Also provided are a culture medium comprising the inhibitor, more particularly selected from the group consisting of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid; 4-Phenyl-1-(3'-sulforpropyl)-1,2,3,6-tetrahydropyridine, cyclohexylsulfamic acid, O-phospho-L-serine, hexafluoroglutamic acid, 8-methoxyquinoline-5-sulfonic acid, 3-amino-2hydroxy-1-propanesulfonic acid, and 3-dimethylamino-1-propanesulfonic acid and 1,2,3,4-tetrahydroisoquinoleine.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/02623

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 7, 8, 10-12, 16-18, 21, 52 (partially)

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid, 3-amino-2-hydroxy-1-propanesulfonic acid, and 3-dimethylamino-1-propanesulfonic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

2. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of DL-2-amino-5-phosphovaleric acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

3. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

4. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of cyclohexylsulfamic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

5. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of O-phospho-L-serine for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

6. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of hexafluoroglutaric acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

7. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 8-methoxyquinoline-5-sulfonic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. Claims: 1,8,10,16,21 (partially) 4,5,13,14,19,20

Use of compounds of formula of claim 4, including 1,2,3,4-tetrahydroisoquinoline for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

9. Claims: 6,9,15,22-44(1)

Process for the preparation of a cell according to claims 22-31, ex vivo cells produced by such process according to claims 35-41, and culture mediums according to claim 32-34, a vessel and kit according to claims 43-44.

10. Claims: 44(2),46-51

Use of an antibody that binds an inhibitor or compound as defined in claim 2,4 or 27, or of a fragment of said antibody that retains the ability to bind the said inhibitor or compound, to identify a substance that can be used to prepare cells for transplantation in a process according to claim 22 or 23 and a method of identifying an inhibitor that can be used to prepare cells for transplantation in a process according to claim 22 or 23, comprising contacting a candidate substance with a mammalian cell and determining whether the candidate substance inhibits the formation of fibrils or causes the breakdown of fibrils, (i) the inhibition of formation of fibrils or (ii) the breakdown of fibrils, indicating that the substance is an inhibitor that can be used in said process, and inhibitors identified by such method (sic).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-21,52. relate to a product/therapeutic application defined by reference to a desirable characteristic or property, namely inhibiting IAPP-associated amyloid deposits.

The claims cover all product/therapeutic application having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such product/therapeutic application. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/therapeutic application by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Present claims 2,11 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the product/therapeutic application relating to the treatment of diabetes (see claim 7) as far as related to the first invention.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02623

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/145 A61K31/662 A61K31/451 A61K31/445 A61K31/47
A61K31/472 A61P3/00 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBASE, BIOSIS, CHEM ABS Data, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 37612 A (PFIZER ;CARTY MAYNARD D (US); KREUTTER DAVID K (US); SOELLER WALTE) 28 November 1996 (1996-11-28) abstract page 2, line 16 -page 4, line 3 ---	1
X	WO 94 22437 A (UNIV KINGSTON) 13 October 1994 (1994-10-13) abstract page 1, line 33 -page 2, line 36 page 4, line 16-32 page 13, line 10-17 page 15, line 21 -page 16, line 2; claim 1 --- -/-	1,2,8, 10,11, 17,21,52

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

10 December 2001

27.03.2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

A. Jakobs

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/02623

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 28187 A (KISILEVSKY ROBERT ;SZAREK WALTER (CA); UNIV KINGSTON (CA); WEAVER) 19 September 1996 (1996-09-19) abstract page 5, line 1 -page 6, line 5 page 12, paragraph 4 -page 14, paragraph 1 page 17, paragraph 1; table 2 ---	1,2,8, 10,11, 17,21,52
X	WO 94 27602 A (CORTEX PHARMA INC) 8 December 1994 (1994-12-08) abstract page 3, line 16 -page 4, line 15 page 4, line 21 -page 6, line 31 page 9, line 23 -page 10, line 25; claim 16; example 16 ---	1,2
X	"USE OF METABOTROPIC AGONISTS IN PROGRESSIVE NEURODEGENERATIVE DISEASES" EXPERT OPINION ON THERAPEUTIC PATENTS, ASHLEY PUBLICATIONS, GB, vol. 5, no. 5, 1995, pages 491-493, XP001012270 ISSN: 1354-3776 the whole document ---	1,2
X	SCHWARCZ R. ET AL: "Excitatory aminoacid antagonists provide a therapeutic approach to neurological disorders." LANCET, (1985) 2/8447 (140-143). CODEN: LANCAO, XP001030857 abstract page 141, column 2, paragraph 3 -page 143, column 1, paragraph 2 ---	1,2
X	WO 93 03714 A (UPJOHN CO) 4 March 1993 (1993-03-04) abstract page 4, line 33 -page 6, line 5 page 11, line 1-25; claims 1-9; tables 3,6,8 --- -/-	1,2,7,8, 10,11, 16,17, 21,52

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/02623

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>COPANI A ET AL: "ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS PROTECTS CULTURED NEURONS AGAINST APOPTOSIS INDUCED BY beta-AMYLOID PEPTIDE" MOLECULAR PHARMACOLOGY, BALTIMORE, MD, US, vol. 5, no. 47, 1995, pages 890-897, XP002079923 ISSN: 0026-895X abstract page 890, column 1-2; figure 3 page 893, column 2, paragraph 2 -page 894, column 1, paragraph 1; figure 5 page 895, column 2, paragraph 1 -page 896, column 1, paragraph 2</p> <p>---</p>	1,2
X	<p>HUTCHINGS R ET AL: "The effect of excitotoxin antagonists on ibotenic acid-induced alteration of APP mRNA hippocampal expression." JOURNAL OF PHARMACY AND PHARMACOLOGY, vol. 47, no. 12B, 1995, page 1131 XP002079922 British Pharmaceutical Conference 1995: Science Proceedings of the 132nd Meeting; Warwick, England, UK; September 15-18, 1995 ISSN: 0022-3573 abstract</p> <p>---</p>	1,2
X	<p>PACHE D M ET AL: "EFFECT OF SELECTIVE EXCITATORY AMINO ACID ANTAGONISTS ON EXCITOTOXIN-INDUCED CHANGES IN APP mRNA EXPRESSION" PHARMACOLOGY REVIEWS AND COMMUNICATIONS, GORDON AND BREACH, CH, vol. 10, no. 3, 1999, pages 205-212, XP001014486 abstract</p> <p>---</p>	1,2
X	<p>WO 98 11923 A (BAYLOR COLLEGE MEDICINE ;GIULIAN DANA J (US)) 26 March 1998 (1998-03-26) the whole document</p> <p>---</p>	1
E	<p>WO 00 71101 A (NEUROCHEM INC ;UNIV KINGSTON (CA)) 30 November 2000 (2000-11-30)</p> <p>the whole document</p> <p>---</p> <p>-/-</p>	1-3,7,8, 10-12, 16-18, 21,52

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/02623

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 64420 A (KONG XIANQI ;NEUROCHEM INC (CA); SZAREK WALTER (CA); UNIV KINGSTON) 2 November 2000 (2000-11-02) abstract page 2, line 15 -page 4, line 25 page 13, line 10 -page 15, line 13 page 22, line 21 -page 23, line 12; claims 1-3,9,12-16,20-22,25-65; examples 3-8 ---	1-3,7,8, 10-12, 16-18, 21,52
X,P	WO 99 40909 A (NEUROCHEM INC) 19 August 1999 (1999-08-19) abstract page 3, line 3-14 page 12, line 7-19 claims 1-12 -----	1-3,7,8, 10-12, 16-18, 21,52

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/02623

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 9637612	A 28-11-1996	US CA EP WO JP JP	6187991 B1 2219629 A1 0827540 A1 9637612 A1 3258024 B2 10507084 T	13-02-2001 28-11-1996 11-03-1998 28-11-1996 18-02-2002 14-07-1998
WO 9422437	A 13-10-1994	CA CA WO WO EP EP EP JP US US US US US	2159326 A1 2159649 A1 9422437 A2 9422885 A1 1060750 A2 0691844 A1 0691976 A1 8508260 T 5643562 A 5972328 A 5728375 A 5840294 A 2001048941 A1	13-10-1994 13-10-1994 13-10-1994 13-10-1994 20-12-2000 17-01-1996 17-01-1996 03-09-1996 01-07-1997 26-10-1999 17-03-1998 24-11-1998 06-12-2001
WO 9628187	A 19-09-1996	US US US AU AU BR CA WO EP JP US US	5643562 A 5972328 A 5840294 A 716218 B2 5097696 A 9607197 A 2213759 A1 9628187 A1 0814842 A1 11501635 T 5728375 A 2001048941 A1	01-07-1997 26-10-1999 24-11-1998 24-02-2000 02-10-1996 11-11-1997 19-09-1996 19-09-1996 07-01-1998 09-02-1999 17-03-1998 06-12-2001
WO 9427602	A 08-12-1994	AU WO US	6836394 A 9427602 A1 5622981 A	20-12-1994 08-12-1994 22-04-1997
WO 9303714	A 04-03-1993	AU AU AU AU CA EP JP WO	664710 B2 2407592 A 3061495 A 3061595 A 2113817 A1 0600973 A1 6510760 T 9303714 A2	30-11-1995 16-03-1993 09-11-1995 09-11-1995 04-02-1993 15-06-1994 01-12-1994 04-03-1993
WO 9811923	A 26-03-1998	US US AU AU EP WO US US	6071493 A 6043283 A 738509 B2 4589497 A 1051195 A1 9811923 A1 2001016326 A1 2001016327 A1	06-06-2000 28-03-2000 20-09-2001 14-04-1998 15-11-2000 26-03-1998 23-08-2001 23-08-2001
WO 0071101	A 30-11-2000	AU AU	4282400 A 4905000 A	10-11-2000 12-12-2000

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/02623

Patent document cited in search report	Publication date		Patent family member(s)		Publication date
WO 0071101	A		WO 0064420 A2		02-11-2000
			WO 0071101 A2		30-11-2000

WO 0064420	A	02-11-2000	AU 4282400 A		10-11-2000
			WO 0064420 A2		02-11-2000
			AU 4905000 A		12-12-2000
			WO 0071101 A2		30-11-2000

WO 9940909	A	19-08-1999	AU 2437899 A		30-08-1999
			CA 2320224 A1		19-08-1999
			EP 1054664 A1		29-11-2000
			WO 9940909 A1		19-08-1999
			JP 2002502871 T		29-01-2002
			US 2002022657 A1		21-02-2002

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 January 2001 (18.01.2001)

PCT

(10) International Publication Number
WO 01/03680 A2

(51) International Patent Classification⁷: **A61K 31/00**

(21) International Application Number: PCT/GB00/02623

(22) International Filing Date: 7 July 2000 (07.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

9916214.1	9 July 1999 (09.07.1999)	GB
60/142,907	9 July 1999 (09.07.1999)	US
9916315.6	12 July 1999 (12.07.1999)	GB
60/142,953	12 July 1999 (12.07.1999)	US

(71) Applicants (for all designated States except US): ISIS INNOVATION LIMITED [GB/GB]; Ewert House, Ewert Place, Summertown, Oxford OX2 7BZ (GB). NEUROCHEM, INC. [CA/CA]; 7220 Frederick-Banting, Suite 100, Montreal, Quebec H4S 2A1 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): CLARK, Anne [GB/GB]; Diabetes Research Laboratories, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE (GB). FRASER, Paul [CA/CA]; Centre for Research into Neurodegenerative Diseases, University of Toronto, 6 Queens Park Crescent West, Toronto M5S 3H2 (CA). VERCHERE, Bruce [CA/CA]; Department of Pathology and Laboratory Medicine, British Columbia, Research Institute for Children and Women's Health, 950 West 28

Avenue, Vancouver, British Columbia (CA). GUPTA, Ajay [CA/CA]; 255 St-Louis, Pointe-Claire, Québec H9R 5L6 (CA). MIGNEAULT, David [CA/CA]; 1125 Haendel, Laval, Québec H7W 3A1 (CA). SZAREK, Walter [CA/CA]; 165 Ontario Street, Suite 301, Kingston, Ontario K7L 2Y6 (CA). WEAVER, Donald [CA/CA]; 27 College View Crescent, Kingston, Ontario K7M 7J8 (CA).

(74) Agents: IRVINE, Claire, Jonquil et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— Without international search report and to be republished upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/03680 A2

(54) Title: COMPOUNDS FOR INHIBITING DISEASES AND PREPARING CELLS FOR TRANSPLANTATION

(57) Abstract: Methods and compositions which are useful in the treatment of amyloidosis. In particular, methods and compositions are provided for inhibiting, preventing and treating amyloid depositions, e.g. in pancreatic islets, wherein the amyloidotic deposits are islet amyloid polypeptide (IAPP)-associated amyloid deposition or deposits. The methods of the invention involved administering to a subject a therapeutic compound which inhibits IAPP-associated amyloid deposits. Accordingly, the compositions and method of the invention are useful for inhibiting IAPP-associated amyloidosis in disorders in which such amyloid deposition occurs, such as diabetes. The invention also provides a process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming fibrils, said process comprising contacting the cells with an inhibitor of fibril formation. In particular the process prepares cells for use in a method of treating diabetes. Also provided are a culture medium comprising the inhibitor and cells for transplantation.

**COMPOUNDS FOR INHIBITING DISEASES AND PREPARING
CELLS FOR TRANSPLANTATION**

Field of the invention

5 The invention relates to compounds for inhibiting amyloid deposits *in vivo*,
cells for transplantation, a process for preparing the cells and a medium for culturing
the cells. In particular the invention relates to the inhibition of islet amyloid
polypeptide (IAPP) deposition *in vivo* and a process for the preparation of islet cells
for transplantation into patients with diabetes.

10

Background to the invention

Amyloidosis refers to a pathological condition characterized by the presence
of amyloid. Amyloid is a generic term referring to a group of diverse but specific
intra- and extracellular protein deposits which are associated with a number of
15 different diseases. The protein deposits comprise largely insoluble fibrillar material.
The deposition of normally soluble proteins in this insoluble form is believed to lead
to tissue malfunction and cell death.

Though diverse in their occurrence, all amyloid deposits have common
morphological properties, including that they stain with specific dyes (e.g. Congo
20 red), and have a characteristic birefringent appearance (sometimes characterized as
"red-green") in polarized light after staining. They also share common ultrastructural
features and common x-ray diffraction and infrared spectra. At least some of the
protein in the deposits is in the form of fibrils. Many different proteins are known to
form fibrils. Such fibrils consist of long cylindrical structures in which the proteins
25 comprise β -sheets that propagate in the direction of the fibril twisting around each
other.

Amyloidosis can be classified clinically as primary, secondary, familial
and/or isolated. Isolated forms of amyloidosis are those that tend to involve a single
organ system compared to systemic amyloidosis involving many organs and tissues.
30 Different amyloids are characterized by the type of protein present in the deposit.
For example, neurodegenerative diseases such as scrapie, bovine spongiform

encephalitis, Creutzfeldt-Jakob disease and the like are characterized by the appearance and accumulation of a protease-resistant form of a prion protein (referred to as AScr or PrP-27) in the central nervous system. Similarly, Alzheimer's disease, another neurodegenerative disorder, is characterized by congophilic angiopathy and neuritic plaques which have the characteristics of amyloid. In this localised form of amyloid the plaque and blood vessel amyloid is formed by the Alzheimer beta protein. Other diseases, such as complications of long-term hemodialysis and sequelae of long-standing inflammation or plasma cell dyscrasias are characterized by the accumulation of amyloid systemically. In each of these cases, a different amyloidogenic protein is involved in amyloid deposition.

Islet amyloid polypeptide (IAPP), also known as "amylin", is known to be capable of forming fibrils which are deposited in the pancreatic islets of patients with Type II diabetes, forming deposits. Once these amyloid deposits have formed, there is no known therapy or treatment which significantly prevents, reduces or clears the deposits *in situ*. The inventors have now identified compounds for this purpose.

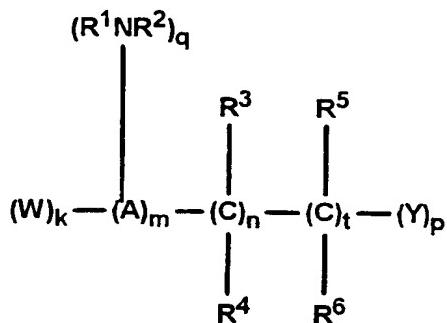
With regard to another aspect of the invention, diseases caused by the death or malfunctioning of a particular type or types of cells can be treated by transplanting into the patient healthy cells of the relevant type of cell. Often these cells are cultured *in vitro* prior to transplantation to increase their numbers, to allow them to recover after the isolation procedure or to reduce their immunogenicity. However, in many instances the transplants are unsuccessful, due to the death of the transplanted cells. The inventors have now also found that culturing of cells can lead to the formation of fibrils from endogenous proteins. Such fibrils are likely to continue to grow after the cells are transplanted and cause death or dysfunction of the cells. The inventors have shown that inhibitors of fibril formation can be used to inhibit the formation of fibrils *in vitro*.

Summary of the invention

The inventors have now identified compounds that can be used to inhibit, reduce or disrupt amyloid deposits *in vivo*. In particular the compounds can be used against amyloid deposits of IAPP *in vivo*. Thus the invention provides methods and

compositions which are useful in the treatment of amyloidosis. In particular, methods and compositions are disclosed for inhibiting, preventing and treating amyloid deposition, for example, in pancreatic islets wherein the amyloidotic deposits to be treated are, in an embodiment, islet amyloid polypeptide (IAPP)-
5 associated amyloid deposits, e.g., having at least some β -sheet structure. The methods of the invention involve administering to a subject a therapeutic compound which inhibits, reduces or disrupts amyloid deposits, e.g., IAPP-associated amyloid deposits. Accordingly, the compositions and methods of the invention are useful for inhibiting amyloidosis in disorders in which such amyloid deposition occurs, such as
10 diabetes.

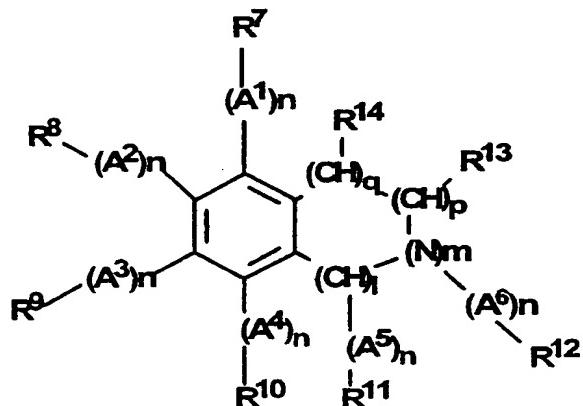
In one embodiment, a method for inhibiting amyloid deposition, particularly IAPP-associated amyloid deposition, in a subject is provided, wherein an effective amount of an IAPP fibril inhibiting compound, or a pharmaceutically acceptable salt thereof, is administered to the subject such that said IAPP-associated amyloid deposition is inhibited. Such compounds include those of the following general
15 formula (a):



wherein k, m, t, p and q are independently 0 or 1; n is an integer from 0 to 3; C is a carbon; N is a nitrogen; W is hydrogen or an anionic group at physiological pH; Y is an anionic group at physiological pH; R^1 and R^2 are independently hydrogen, alkyl, an anionic group at physiological pH, or R^1 and R^2 , taken together with the nitrogen to which they are attached, may form an unsubstituted or substituted heterocycle having from 3 to 7 atoms in the heterocyclic ring; R^3 is hydrogen, halogen, thiol or hydroxyl; R^4 , R^5 and R^6 are independently hydrogen or halogen; and A is hydrogen or C_1 to C_6 alkyl; or a pharmaceutically acceptable ester, acid or salt thereof.
20
25
30

Preferred therapeutic compounds include 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid; 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine; cyclohexylsulfamic acid; *O*-phospho-L-serine; hexafluoroglutaric acid; 8-methoxyquinoline-5-sulfonic acid; 3-amino-2-hydroxy-1-propanesulfonic acid; and 3-dimethylamino-1-propanesulfonic acid, and pharmaceutically acceptable esters, acids or salts thereof.

In another embodiment a method for inhibiting amyloid deposition, particularly IAPP-associated amyloid deposition, in a subject is provided, wherein an effective amount of an IAPP fibril inhibiting compound, or a pharmaceutically acceptable ester, acid or salt thereof, is administered to the subject such that said IAPP-associated amyloid deposition is inhibited. Such compounds include those of the following general formula (b):



wherein C is a carbon; N is a nitrogen; H is a hydrogen; A¹, A², A³, A⁴, A⁵ and A⁶ are independently alkyl, O, S, or -NH; m and n (for each individual A group) are independently 0 or 1; p, q and l are independently 0, 1, or 2; R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², and each R¹⁴ are independently hydrogen, alkyl, alicyclyl, heterocycyl or aryl, each R¹³ is independently hydrogen, alkyl, alicyclyl, heterocycyl, aryl or an anionic group, and adjacent R groups (e.g., R⁷ and R⁸) may form an unsubstituted or substituted cyclic or heterocyclic ring.

Preferred therapeutic compounds include 1,2,3,4-tetrahydroisoquinoline. In

another embodiment the invention relates to a method for reducing IAPP-associated amyloid deposits in a subject having IAPP-associated amyloid deposits, the method comprising administering to a subject an effective amount of an IAPP fibril inhibiting compound, or a pharmaceutically acceptable salt thereof, such that IAPP-associated amyloid deposits are reduced.

The therapeutic compounds of the invention are administered to a subject by a route which is effective for inhibiting IAPP-associated amyloid deposition. Suitable routes of administration include oral, transdermal, subcutaneous, sublingual, buccal, intravenous and intraperitoneal injection. The therapeutic compounds can be administered with a pharmaceutically acceptable vehicle.

The invention further provides pharmaceutical compositions for treating amyloidosis. The pharmaceutical compositions include a therapeutic compound of the invention in an amount effective to inhibit IAPP-associated amyloid deposition, and a pharmaceutically acceptable vehicle.

The inventors have also found that the culturing of cells *in vitro* can lead to the formation of fibrils from endogenous proteins. Since the process is progressive, the fibrils are likely to continue to grow after the cells are transplanted and cause the death or dysfunction of the cells. This may occur even when the cells are from a healthy donor and when the patient receiving the transplant does not have a disease that is characterised by the presence of fibrils. The inventors have shown that the culturing of islet cells from a non-diabetic donor for the purpose of transplantation into a patient with type I diabetes leads to the formation of fibrils in cell clusters *in vitro*. They have also shown that inhibitors of fibril formation can be used to reduce the formation of fibrils *in vitro*.

Thus the invention also provides a process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming fibrils, said process comprising contacting the cells with an inhibitor of fibril formation. Any inhibitor of fibril formation may be used, including any such compounds mentioned herein.

The invention also provides a culture medium or culture medium pre-mix that comprises an inhibitor of the invention. The invention further provides *ex vivo* cells

made by the process of the invention. The invention provides the *ex vivo* cells for use in a method of treatment of the human or animal body by therapy. In particular the invention provides *ex vivo* cells of the invention which are islet cells for use in a method of treating diabetes.

5

Brief description of the drawings

Figure 1 shows the effect of candidate substances on the proportion of non-fibrillar IAPP in a mixture of fibrils and non-fibrillar IAPP at time 0 and after incubation over a period of 24 h. The unshaded columns show results at time 0 and the shaded columns show results at time 24 h. The vertical axis shows the proportion of non-fibrillar IAPP in the supernatent (as a percentage of control). Changes in the direction of the arrow represent a relative increase in non-fibrillar IAPP (decrease in fibrils).

10

Figure 2 shows the effect of polyvinylsulfonate (A) and compound vi (C), compared to IAPP alone (B), on formation of fibrils. The vertical axis shows fluorescence units. The horizontal axis shows time in hours.

15

Figure 3 shows the effects of compounds i, iii, x and iv on molecular conformation of the peptide as measured by circular dichroic spectroscopy at time 0 (left) and time 24 h (right). The horizontal axis shows the wave length.

20

Figures 4a and 4b show respectively isolated human and mouse islet cells which have been cultured for 6 days in glucose and RPM1. Amyloid fibrils can be seen between the cells (as shown by arrows). The dots show fibrils which have been immunogold labelled for IAPP.

25

Figure 5 shows the fibrils formed by IAPP alone or in the presence of candidate substances.

Figure 6 shows mean islet survival in the presence of the compounds. (A) represents guandinoethanosulfonic acid.

30

Detailed description of the invention

The present invention will be more fully illustrated by reference to the definitions set forth below.

"Amyloid" includes IAPP-associated amyloid, including, but not limited to, β -sheet amyloid assembled substantially from IAPP subunits. "Inhibition" of amyloid deposition includes preventing or stopping of IAPP-associated amyloid formation, inhibiting or slowing down of further IAPP-associated amyloid deposition in a subject with ongoing amyloidosis, e.g., already having amyloid deposits, and reducing or reversing IAPP-associated amyloid deposits in a subject with ongoing amyloidosis. Inhibition of amyloid deposition is determined relative to an untreated subject, or relative to the treated subject prior to treatment, or, e.g., determined by clinically measurable improvement in pancreatic function in a diabetic patient.

Pharmaceutically acceptable salts of the therapeutic compound, where applicable, are within the scope of the invention, e.g., alkali metal, alkaline earth metal, higher valency cation (e.g., aluminum salt), polycationic counter ion or ammonium salts. Where a compound is anionic, a preferred pharmaceutically acceptable salt is a sodium salt. Other salts are also contemplated, e.g., HCl, citric acid, tartaric acid salts, within their pharmaceutically acceptable ranges.

The therapeutic compound of the invention can be administered in a pharmaceutically acceptable vehicle. As used herein "pharmaceutically acceptable vehicle" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like which are compatible with the activity of the compound and are physiologically acceptable to the subject. An example of a pharmaceutically acceptable vehicle is buffered normal saline (0.15 molar NaCl). Except insofar as any conventional media or agent is incompatible with the therapeutic compound, use thereof in the compositions suitable for pharmaceutical administration is contemplated. Supplementary active compounds can also be incorporated into the compositions.

An "anionic group," as used herein, refers to a group that is negatively charged at physiological pH (for example at pH 6.6) or at a pH at which mammalian cells can be cultured (for example any such pH ranges mentioned herein). Preferred

anionic groups include carboxylate, sulfate, sulfonate, sulfinate, sulfamate, tetrazolyl, phosphate, phosphonate, phosphinate, and phosphorothioate or functional equivalents thereof. "Functional equivalents" of anionic groups include bioisosteres, e.g., bioisosteres of a carboxylate group. Bioisosteres encompass both classical
5 bioisosteric equivalents and non-classical bioisosteric equivalents. Classical and non-classical bioisosteres are known in the art (see, e.g., Silverman, R.B. *The Organic Chemistry of Drug Design and Drug Action*, Academic Press, Inc.: San Diego, CA, 1992, pp. 19-23). A particularly preferred anionic group is a carboxylate.

The term "alkyl" includes saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C₁-C₃₀ for straight chain, C₃-C₃₀ for branched chain), and more preferably has 20 or fewer carbon atoms in the backbone. Likewise, cycloalkyls may have from 4-10 carbon atoms in their ring structure, more preferably have 5, 6 or 7 carbons in the ring structure.
10
15

Moreover, the term alkyl includes both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, e.g., with the substituents described above. An "aralkyl" moiety is an alkyl substituted with an aryl (e.g.,
20
25
30

phenylmethyl (benzyl)).

The term "aryl" herein includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, tetrazole, pyrazole, 5 pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles", "heteroaryls" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, 10 as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, 15 arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g., tetralin).

20 The terms "alkenyl" and "alkynyl" include unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" means an alkyl group, as defined above, but having from one to ten carbons, more preferably 25 from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Preferred alkyl groups are lower alkyls.

30 The terms "heterocyclyl" or "heterocyclic group" include 3- to 10- membered ring structures, more preferably 4- to 7- membered rings, which ring structures include one to four heteroatoms. Heterocyclyl groups include pyrrolidine, oxolane, thiolane, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones

and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxy carbonyl, aminocarbonyl, 5 alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulphydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, 10 or an aromatic or heteroaromatic moiety.

The terms "polycyclyl" or "polycyclic group" include two or more cyclic rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed 15 "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy carbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxy carbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, 20 dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulphydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, or an aromatic or heteroaromatic moiety.

25 The term "heteroatom" includes an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

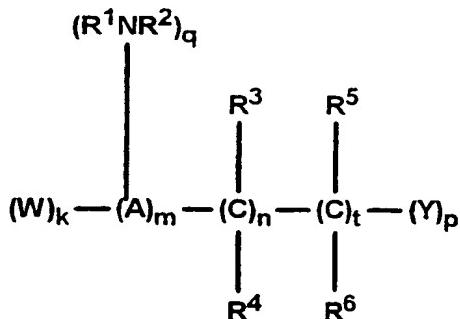
The term "aryl aldehyde," as used herein, includes compounds represented by the formula Ar-C(O)H, in which Ar is an aryl moiety (as described above) and -C(O)H is a formyl or aldehydo group.

30 It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the

isomers arising from such asymmetry (e.g., all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis. Furthermore, alkenes can include either the E- or Z- geometry, where appropriate.

The present methods and compositions, in embodiments, inhibit, prevent and treat amyloid deposition in pancreatic islets wherein the amyloidotic deposits to be treated are islet amyloid polypeptide (IAPP)-associated amyloid deposits, e.g., having at least some β -sheet structure. The methods of the invention include administering to a subject a therapeutic compound which inhibits, reduces or disrupts IAPP-associated amyloid deposits. Accordingly, the compositions and methods of the invention are useful for inhibiting amyloidosis in disorders in which such amyloid deposition occurs, such as diabetes.

In one embodiment, a method for inhibiting IAPP-associated amyloid deposition in a subject is provided, wherein an effective amount of an IAPP fibril inhibiting compound, or a pharmaceutically acceptable salt thereof, is administered to the subject such that said IAPP-associated amyloid deposition is inhibited. Such compounds include those of the following general formula (a)

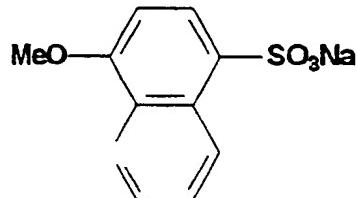
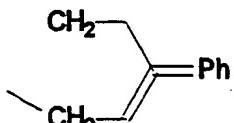
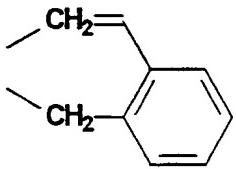


wherein k, m, t, p and q are independently 0 or 1; n is an integer from 0 to 3; C is a carbon, N is a nitrogen; W is hydrogen or an anionic group at physiological pH; Y is an anionic group at physiological pH; R¹ and R² are independently hydrogen, alkyl, an anionic group at physiological pH, or R¹ and R², taken together with the nitrogen to which they are attached, may form an unsubstituted or substituted heterocycle having from 3 to 7 atoms in the heterocyclic ring; R³ is hydrogen, halogen, thiol or

hydroxyl; R⁴, R⁵, and R⁶ are independently hydrogen or halogen; and A is hydrogen or C₁ to C₆ alkyl; or a pharmaceutically acceptable salt thereof.

In an embodiment, W is preferably -COOH; Y is preferably -COOH, -SO₃H, -PO₃H₂ or -OP(O)(OH)₂; R¹ is preferably H, Me or hydroxypropyl; R² is preferably H, Me or -SO₃H; R³ is preferably H, F, or OH; when R¹ and R², taken together with the nitrogen to which they are attached, form an unsubstituted or substituted heterocycle, preferred groups include

10



15

R⁴, R⁵ and R⁶ are preferably H or F; A is preferably H, CH, CF₂ or alkyl which may be substituted or unsubstituted, straight, branched or cyclic, e.g., cyclohexyl.

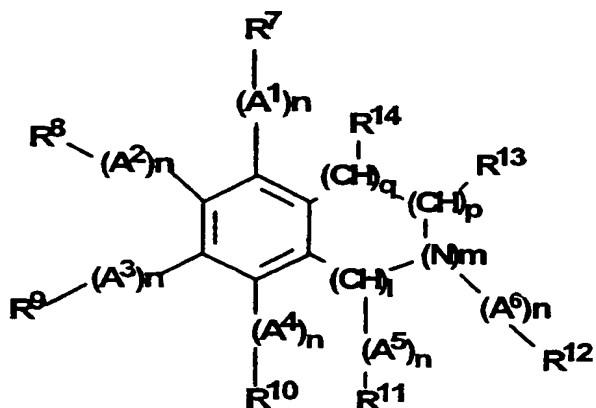
20

Preferred therapeutic compounds include 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid; 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine; cyclohexylsulfamic acid; O-phospho-L-serine; hexafluoroglutaric acid; 8-methoxyquinoline-5-sulfonic acid; 3-amino-2-hydroxy-1-propanesulfonic acid; and 3-dimethylamino-1-propanesulfonic acid, and pharmaceutically acceptable salts thereof.

25

In another embodiment a method for inhibiting IAPP-associated amyloid deposition in a subject is provided, wherein an effective amount of an IAPP fibril inhibiting compound, or a pharmaceutically acceptable salt thereof, is administered to the subject such that said IAPP-associated amyloid deposition is inhibited. Such compounds include those of the following general formula (b)

-13-



wherein C is a carbon; N is a nitrogen; H is a hydrogen; A¹, A², A³, A⁴, A⁵ and A⁶ are independently alkyl, O, S, or -NH; m and n (for each individual A group) are independently 0 or 1; p, q and l are independently 0, 1, or 2; R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², and each R¹⁴ are independently hydrogen, alkyl, alicyclyl, heterocycyl or aryl, each R¹³ is independently hydrogen, alkyl, alicyclyl, heterocycyl, aryl or an anionic group, and adjacent R groups (e.g., R⁷ and R⁸) may form an unsubstituted or substituted cyclic or heterocyclic ring.

Preferred therapeutic compounds include 1,2,3,4-tetrahydroisoquinoline.

20 A further aspect of the invention includes pharmaceutical compositions for treating amyloidosis. The therapeutic compounds in the methods of the invention, as described hereinbefore, can be incorporated into a pharmaceutical composition in an amount effective to inhibit amyloidosis or reduce amyloid deposits, in a pharmaceutically acceptable vehicle.

25 In the methods of the invention, amyloid deposition in a subject is inhibited by administering a therapeutic compound of the invention to the subject. The term subject includes living organisms in which amyloidosis can occur. Examples of subjects include humans, apes, monkeys, cows, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof, as well as cells therefrom, e.g., islet cells, in culture.

30 Administration of the compositions of the present invention to a subject to be treated can be carried out using known procedures, at dosages and for periods of time

effective to inhibit amyloid deposition or reduce amyloid deposits in the subject. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the amount of amyloid already deposited at the clinical site in the subject, the age, sex, and weight of the subject, and the ability of the therapeutic compound to inhibit amyloid deposition or reduce amyloid deposits in the subject. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active compound may be administered by routes such as oral, sublingual, buccal, transdermal, subcutaneous, intravenous, and intraperitoneal administration. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids, enzymes and other natural conditions which may inactivate the compound.

The compounds of the invention can be formulated to ensure proper distribution *in vivo*. For example, the therapeutic compounds of the invention can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs ("targeting moieties"), thus providing targeted drug delivery (see, e.g., V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134); gp120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273. In a preferred embodiment, the therapeutic compounds of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety.

To administer the therapeutic compound by other than parenteral

administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the therapeutic compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.*, (1984) *J. Neuroimmunol.* 7:27).

The therapeutic compound may also be administered parenterally, sublingually, buccally, intraperitoneally, intraspinally, or intracerebrally. Dispersions can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The vehicle can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

Sterile injectable solutions can be prepared by incorporating the therapeutic

compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the therapeutic compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yield a powder of the active ingredient (i.e., the therapeutic compound) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic compound and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the therapeutic compound may be incorporated with excipients and used in the form of ingestible tablets, sublingual/buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the therapeutic compound in the compositions and preparations may, of course, be varied. The amount of the therapeutic compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the treatment of amyloid deposition in subjects.

Active compounds are administered at a therapeutically effective dosage sufficient to inhibit amyloid deposition in a subject. A "therapeutically effective

"dosage" preferably inhibits amyloid deposition and/or reduces amyloid deposits by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects or to the same subject prior to treatment.

5 The ability of a compound to inhibit amyloid deposition or reduce amyloid deposits can be evaluated in an animal model system that may be predictive of efficacy in inhibiting amyloid deposition or reducing amyloid deposits in human diseases. The ability of a compound to inhibit amyloid deposition can also be evaluated by examining the ability of the compound to inhibit amyloid deposition *in vitro* or *ex vivo*, e.g., using an ELISA assay. The effect of a compound on the secondary structure of the amyloid can be further be determined by circular dichroism (CD) or infrared (IR) spectroscopy.

10

15 CD and IR spectroscopy are particularly useful techniques because the information obtained is a direct measure of the ability of a test compound to prevent or reverse amyloidosis, by determining the structural effect of a compound on amyloid protein folding and/or fibril formation. This contrasts with previously known methods which measure cellular trafficking of amyloid protein precursors or interactions between amyloid and extracellular matrix proteins, providing only indirect evidence of potential amyloid-inhibiting activity. It should further be noted that CD and IR spectroscopy can also detect compounds which cause an increase in, e.g., β -sheet folding of amyloid protein, and thereby stabilize the formation of amyloid fibrils.

20

25 The deposition of amyloid is a multi-stage process. Accordingly, an agent useful for treating amyloidosis has many potential modes of action. An agent which inhibits amyloid deposition could act in one or more of the following ways, which are shown by way of illustration and not limitation:

1. Inhibition or delay of protein folding in solution
 2. Inhibition or delay of aggregation of amyloid peptides into fibrils and/or deposits
 3. Disruption/dissolution/modification of amyloid fibrils and/or deposits
- 30 Categories 1 and 2 correspond to prevention of the formation of amyloid

deposits (slowing down or halting amyloid deposition), and category 3 corresponds to removal or modification of deposits already formed (removal or reduction of existing amyloid deposits).

In another aspect the process of the invention aims to reduce the amount of amyloid deposits that are present in a cell preparation before transplantation. As mentioned amyloid deposits will comprise at least some protein present in the form of fibrils.

Such fibrils typically have an ordered and repeating structure created by the regular assembly of the protein components. Typically a fibril is straight and unbranched. It is generally insoluble in the cytoplasm or in extracellular compartments. The fibril may or may not be insoluble in distilled water, or organic solvents, such as hexafluoroisopropanol or trifluoroethanol. It typically has a diameter of 5 to 20 nm, for example 7 to 15 or 10 to 12 nm. The protein in the fibril generally forms one, two or more β -strands which are typically oriented substantially perpendicular to the long axis of the fibril and may form β -sheets that propagate substantially in the direction of the fibril twisting around each other. Fibrils are generally in the form of small linear aggregates of molecules in β -sheet construction or of filamentous structure of varying extended lengths.

Typically the protein that forms the fibril has at least 30%, such as at least 50 or 70% of its native structure as β -sheet. In the case of certain fibrils the native form of the protein (i.e. the soluble non-fibril form) has an α -helical region, which may in all or part be converted to a β -sheet structure in the fibril. The protein is typically a secreted extracellular protein, but may be an intracellular protein. The protein may be the wild-type or an alternative form, such as a mutated form. The alternative form can be a truncated form of the wild-type protein.

The protein is typically IAPP, A β peptide (involved in Alzheimer's disease), prion protein, immunoglobulin light chain, amyloid A protein, transthyretin, cystatin, β 2-microglobulin, apolipoprotein A-1, gelsolin, calcitonin, atrial natriuretic factor, lysozyme variants, insulin, or fibrinogen.

The protein may be one which has sequence or structural homology with any of these particular proteins. Preferably the protein has sequence or structural

homology with IAPP. The protein may be one which does not contain any tryptophan residues in its sequence.

The inhibitor of fibril formation is able to reduce the amount of fibril formation that occurs in conditions in which fibril formation would occur. Thus an inhibitor can be identified in an assay by contacting a candidate substance with a protein that forms fibrils under conditions in which fibril formation would occur and determining whether fibril formation is inhibited by the substance. In one embodiment the inhibitor may interact with preformed fibrils to modulate their architecture resulting in the breaking of the fibrils into monomeric or small oligomeric peptide components. The protein may be any of the proteins mentioned herein.

In the *in vitro* assay, changes in the proportion of monomeric/small oligomeric protein components in a mixture of protein and fibrils can be measured by assaying the non-fibrillar components. The effects of candidate substances on fibril formation can also be measured using thioflavine T spectroscopy or circular dichroic spectroscopy. Circular dichroic spectroscopy indicates the effect of the candidate substances on the molecular conformation of the soluble or other forms of non-fibrillar peptide. In particular, conversion of the molecule to β -conformation which indicates β -sheet formation.

In the assay the inhibitor typically inhibits fibril formation by at least 10%, at least 20%, at least 30%, at least 40% at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% or at least 99.9% at a concentration of the inhibitor of 10ng ml^{-1} , 100ng ml^{-1} , $1\mu\text{g ml}^{-1}$, $10\mu\text{g ml}^{-1}$, $100\mu\text{g ml}^{-1}$, $500\mu\text{g ml}^{-1}$, 1mg ml^{-1} , 10mg ml^{-1} or 100mg ml^{-1} , or a molarity of inhibitor of 100nM , $1\mu\text{M}$, $10\mu\text{M}$, $100\mu\text{M}$, 1mM , 10mM or 100mM . In one embodiment such effects are measured over 24 hours in an assay in which the concentration of the monomeric protein is $20\mu\text{M}$. The percentage inhibition represents the percentage decrease in amount of fibril formation in a comparison of assays in the presence and absence of the test substance. Any combination of the above mentioned degrees of percentage inhibition and concentration of inhibitor may be used to define an inhibitor of the invention, with greater inhibition at lower concentrations being preferred.

An inhibitor of IAPP fibril formation typically causes any of the above percentage inhibition at any of the above concentrations or molarities when contacted with an 80 µg/ml solution of human IAPP in distilled water, at 25°C, over 24 hours.

The inhibitor is typically non-toxic towards the cells (e.g. in culture), for example at any of the concentrations mentioned above. The inhibitor may be non-toxic towards any of the mammals mentioned herein, and thus maybe pharmaceutically acceptable. The inhibitor may or may not be able to enter the cells, typically by diffusing across the cell membrane.

The inhibitor may prevent the neoformation and/or growth of fibrils and/or may breakdown any preformed fibrils which are present. The inhibitor typically binds to the monomeric form of the protein and prevents it oligomerising to form the fibril. The inhibitor may bind to the multimeric form to prevent further protein binding and/or modulate the structure of the multimeric form to cause breakdown of the multimeric form into its component peptide fragments. Such a binding may be reversible or non-reversible. The binding may cause a change in the structure of the monomeric or multimeric form of the protein and/or a change in the structure of the inhibitor. In one embodiment the surface of the inhibitor mimics the part of the surface of the monomeric form which will bind the multimeric form.

The inhibitor may be congo red (e.g as described in WO 94/01116), an acridinone or related molecule (e.g. as described in WO 97/16191), a naphthylazo compound (e.g. as described in WO 97/16194), hexadecyl-N-methylpiperidinium bromide (*J. Biol. Chem.* (1986) **271** (8), 4086-4092), or a saccharide or saccharide composition (e.g. as described in WO 99/0999). The inhibitor may be a peptide or a substituted peptide as described in *J. Am. Chem. Soc.* (1998) **120**, 7655-7656. The inhibitor may be an organic solvent (e.g. dimethylsulphoxide or polyethyleneglycol).

The inhibitor may be of the formula (a) or (b), including any of the specific embodiments or specific compounds covered by (a) or (b) which are discussed above.

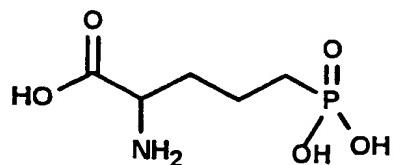
Preferably the inhibitor is any one of compounds (i) to (x) below or a pharmaceutically acceptable salt thereof:

- (i) 3-(3-hydroxy-1-propyl) amino-1- propanesulfonic acid



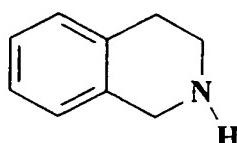
(ii) DL-2-amino-5-phosphovaleric acid

5



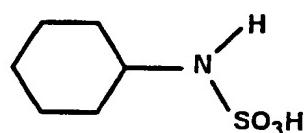
(iii) 1, 2, 3, 4 - tetrahydroisoquinoline

10



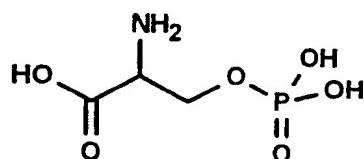
(iv) Cyclohexylsulfamic acid

15



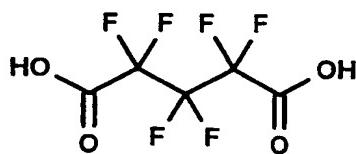
(v) O-Phospho-L-serine

20



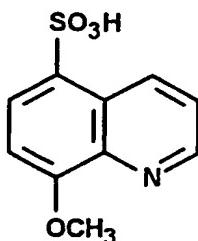
(vi) Hexafluoroglutamic acid

25



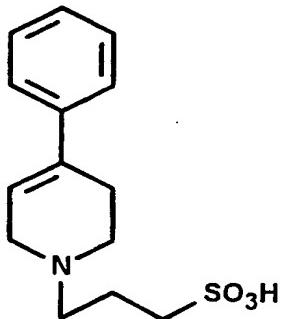
(vii) 8-methoxyquinoline-5-sulfonic acid

30

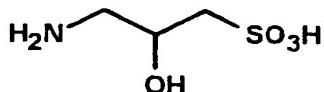


(viii) 4-phenyl-1-(3' sulfopropyl)-1,2,3,6-tetrahydropyridine

5



10 (ix) 3-amino-2-hydroxy-1-propanesulfonic acid



15 (x) 3-dimethylamino-1-propanesulfonic acid



	Compound No.	Form of Compound	CAS Number	Supplier
20	i		58431-88-2	Neurochem Inc.
	ii		76326-31-3	Sigma-Aldrich
	iii	Hydrochloride	14099-81-1	Sigma-Aldrich
	iv	Sodium salt	139-05-9	Sigma-Aldrich
	v		407-41-0	Sigma-Aldrich
	vi		376-73-8	Sigma-Aldrich
25	vii		40712-20-7	Sodium salt available from Neurochem Inc.
	viii	Sodium salt		Neurochem Inc.
	ix		7013-33-4	Neurochem Inc.
	x		29777-99-9	Neurochem Inc.

In the discussion below the term 'specific inhibitor' includes any of the compounds discussed herein, such as those described by either of the general formulae (a) or (b) or their salts (including (i) to (x) above as well as their salts discussed above). The inhibitor of the invention may be structurally and/or functionally equivalent to any of the specific inhibitors. Thus the inhibitor of the invention may be capable of competing with any of the specific inhibitors to bind the monomeric or multimeric form of the protein. Thus the inhibitor may bind the monomeric or multimeric form at the same place as any of the specific inhibitors.

5 The inhibitor may mimic the surface of any of the specific inhibitors. Thus the inhibitor of the invention may bind to an antibody that binds to the specific inhibitors, and thus may be capable of inhibiting the binding of the antibody to the specific inhibitor. Such an antibody can be made by known methods, including administering the specific inhibitor to an animal in association with a carrier to make it more immunogenic. The inhibitor may mimic the shape, size, flexibility or

10 electronic configuration of any of the specific inhibitors. It is typically a derivative of any of the specific inhibitors.

15

The antibody may be used to identify inhibitors from libraries of compounds, such as combinatorial libraries. Alternatively inhibitors which mimic the specific inhibitors may be designed computationally and made by synthetic chemistry techniques.

20 The cells of the invention are generally going to be transplanted into a patient suffering from a disease. The disease may or may not be a disease in which fibrils are present in the patient. In the case of diseases in which fibrils are present in the patient the fibrils may cause the disease, or at least some of the symptoms of the disease. The fibrils may be formed because of the disease, and may exacerbate the symptoms of the disease. The disease may be type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen α -chain amyloidosis.

25

30

In the disease fibrils may form in a particular tissue, and thus may affect a particular type or types of cells. The fibrils may be made of a protein produced by such cells. Alternatively the fibrils may be made of a protein which is produced in a different type of cell or tissue. The fibrils may be located in an intracellular compartment of the cells, such as in the cytoplasm or cytoplasmic organelles.

5 Alternatively the fibrils are located in the extracellular compartment, for example in the proximity of or contacting the cells, which have produced the protein from which the fibrils are made.

10 Alternatively in the disease the cells may be affected by other factors which are not connected with fibrils, for example by an autoimmune attack or pathogenic infection, such as a bacterial or viral infection. The disease may be type I diabetes. Such cells being affected in the disease may be the same as or different from the cells discussed herein. The cells of the invention are capable of forming fibrils or susceptible to the deleterious effect of fibrils.

15 Typically fibrils damage cells by causing a decrease in the amount of substances produced (or secreted) by the cells, or kill the cells or induce apoptosis and cell death.

20 Thus the *ex vivo* cells of the invention are typically of the same or similar type as the cells which have been affected by the disease. The process of the invention is applicable to cells that can form fibrils after transplantation, which as discussed above typically also form fibrils when in culture. In one embodiment the *ex vivo* cells are in a preparation that comprises the inhibitor.

25 The cells are mammalian cells, such as human, primate, rodent, rabbit, ovine, porcine, bovine, feline or canine cells. The cells are typically cells that naturally express a protein that is capable of forming a fibril. In one embodiment the cells are endocrine cells. The cells may be islet, liver, muscle, kidney or neuronal cells. In one embodiment the cells are genetically modified, and are, for example, capable of expressing genes not naturally expressed by the cell. In one embodiment the cells are islet cells and the fibrils comprise human islet amyloid polypeptide.

30 The cells of the invention are typically from a donor who generally does not have the relevant disease. In one embodiment the cells are from the patient. The

cells may be taken from the patient to increase their numbers in vitro and/or the cells may be treated therapeutically in some manner before administering them back to the patient. For example the cells may be treated with agents, which act against (e.g. kill) pathogens.

5 The cells can be obtained from the donor or patient by standard techniques. Before being cultured the cells are generally further purified, for example using collagenase dissociation and/or density gradient centrifugation techniques or cell sorting techniques.

10 The cells are contacted with the inhibitor before transplantation. Generally the cells are contacted with the inhibitor when being cultured. Thus in the process the cells are cultured in the presence of the inhibitor. However, in one embodiment the cells are contacted with inhibitor after culturing, but before transplantation, for example in the case of inhibitors which cause breakdown of pre-existing fibrils. In one embodiment of the process the fibrils are initially present in the said cells prior to 15 contacting with said inhibitor and said inhibitor causes breakdown of said fibrils.

The inhibitor may cause breakdown of some or of all the fibrils present.

20 The cells are typically cultured in order to allow them to recover after the isolation procedure, to increase their numbers before transplantation, to treat them therapeutically in some manner or to change the proportion of the different types of cells present in the culture. In order to achieve this last aim the cells may be cultured in conditions which allow the survival of certain types of cells over other types of cells. In one embodiment the cells are cultured in conditions which reduce the numbers of 'passenger' leukocytes in order to reduce the immunogenicity of the 25 cells.

30 Typically cells are cultured for from 12 to 150 hours, for example from 24 to 100 hours, before transplantation. Generally the cells are cultured at from 20°C to 45°, for example 30°C to 40°C, preferably 35°C to 37°C. Generally the pH of the culture is from 6.6 to 8.0, preferably 7 to 7.6 or 7.2 to 7.4. Thus the cells of the invention may be cells which have been cultured under such conditions and/or in the culture medium discussed below.

Generally the cells are cultured in the culture medium of the invention which

-26-

comprises an inhibitor of the invention. Such a medium is capable of providing support for any of the cells of the invention. Thus the medium will provide substances to keep the cells alive, and may also allow growth and replication of the cells. The medium thus comprises nutrition for the cells. The nutrition will be in the 5 form of an assimilable carbon source, such as a carbohydrate source or amino acids. Thus the medium may comprise sugars, such as glucose, fructose, mannose or galactose or non-sugar carbohydrates, such as lactate or pyruvate. These may be present at from 1 to 40 mM, such as 10 to 30 mM. The medium may comprise an amino acid, such as arginine, cysteine, histidine, isoleucine, leucine, lysine, 10 methionine, phenylalanine, threonine, tryptophan, tyrosine or valine.

The medium comprises water. The medium may comprise ions from inorganic salts, such as sodium, potassium, calcium, magnesium, iron, selenium, carbonate, phosphate or sulphate ions. The medium may comprise vitamins, such as nicotinamide. The medium may comprise xanthine.

15 The medium may comprise growth factors or hormones. The medium may comprise proteins, such as binding and transport proteins, for example transferrin or albumin. The medium may comprise lipids, insulin or ethanolamine.

Some of the above components may be provided in the medium by an extract 20 from an animal or equivalent supplements. Thus the medium may comprise such an extract. The extract may be partially purified. The extract may be serum, such as foetal calf serum.

The medium may comprise antibiotics, such as penicillin.

25 The medium may comprise the constituents of the commercially available media which can be obtained from NBLTM or GibcoTM, such as RPMI 1640TM, Dulbecco's modified Eagle's mediumTM, Medium 199TM, CMRL 1066TM.

In the medium the inhibitor is typically present at a concentration of 1 to 10,000 µM, for example 10 to 1000 µM or 100 to 500 µM.

30 The culture medium pre-mix typically has the same constituents as the culture medium but contains less water, such as less than from 50%, 10%, 1% or 0.1% of the water present in the culture medium. The pre-mix may be in the form of a liquid, gel or powder. Typically the pre-mix can be converted to a culture medium by adding

water.

The culture medium may comprise a precursor of the inhibitor which provides the inhibitor when contacted with any of the cells discussed above. The pre-mix may comprise a precursor which provides the inhibitor when contacted with water or with the cells. The term 'inhibitor' includes such precursors.

5 The culture of the invention comprises the cells of the invention and culture medium of the invention. The culture comprises at least 100 cells, such as at least 10^3 , 10^5 , 10^7 or 10^9 cells

10 The inhibitor may also be administered to a patient who has received a transplant of the cells of the invention. The inhibitor is administered to prevent damage *in vivo* to the transplanted cells by fibrils. Thus the invention provides an inhibitor for use in inhibiting fibril formation by, or breaking fibrils down in, a transplanted cell preparation.

15 The invention also provides a vessel for containing a culture of cells, which vessel is coated with the inhibitor. The vessel may be a Petri dish or a flask. The vessel may comprise glass or plastic. The inhibitor is generally present on the surface which will be in contact with the culture. The inhibitor is present in a form in which it is capable of being released into the culture when the culture comes into contact with it.

20 The invention also provides a kit for culturing cells comprising the culture medium or pre-mix of the invention or a vessel of the invention.

25 Antibodies that bind to the inhibitors of the invention may be used to screen for inhibitors based on their ability to bind the antibody. Typically such a screening is carried out on a library of candidate compounds. Thus the invention provides use of an antibody that binds an inhibitor of the invention, or of a fragment that retains the ability of said antibody to bind said inhibitor, to identify a compound that can be used to prepare cells for transplantation in the process of the invention.

30 Inhibitors can also be identified based on their ability to inhibit the formation of fibrils or breakdown fibrils in a cell preparation that comprises any of the types of cells mentioned herein which can be treated in the process of the invention. Thus the invention provides a method of identifying an inhibitor that can be used to prepare

5 cells for transplantation in a process of the invention, comprising contacting a candidate substance with a mammalian cell and determining whether the candidate substance inhibits the formation of fibrils or causes the breakdown of fibrils, (i) the inhibition of formation of fibrils or (ii) the breakdown of fibrils, indicating that the substance is an inhibitor that can be used in said process. The cell is typically a human islet cell.

10 The inhibitor may also identified based on its ability to breakdown fibrils or to inhibit the formation of fibrils. Thus the invention provides a method of identifying an inhibitor that can be used to prepare cells for transplantation in a process of the invention, comprising contacting a candidate substance with a protein capable of forming fibrils, or with a fibril, and determining whether the substance inhibits the formation of the protein into a fibril, or whether the substance causes the breakdown of the fibril, (i) inhibition of fibril formation or, (ii) the breakdown of fibrils, indicating that the substance can be used in said process. The protein is typically human islet amyloid peptide or the fibrils are typically made of human islet amyloid peptide.

15 The invention also provides an inhibitor identified in the use and method of the invention. Such an inhibitor may be used in any of the aspects of the invention discussed herein, or may be used in a method of treatment of the human or animal body by therapy. Thus the invention also provides a process, culture medium premix, *ex vivo* cell, pharmaceutical composition vessel or kit of the invention wherein the inhibitor is an inhibitor that has been identified in the use or method of the invention.

20 A therapeutically effective number of cells of the invention may be administered to a human or animal in need of treatment. Diseases which may be treated using the cells of the invention are those in which a particular cell type is malfunctioning or has died. The condition of a patient suffering from such a disease can thus be improved.

25 Thus the invention provides cells of the invention for use in a method of treatment of the human or animal body by therapy, in particular for use in a method of treating diabetes. The invention also provides use of any of the cells of the

invention in the manufacture of a medicament for the treatment of a disease in which a particular cell type is malfunctioning or has died, in particular for the treatment of diabetes. Thus the invention provides a method of treating a disease in which a particular cell type is malfunctioning or has died comprising administering a cell of the invention to an individual with the disease.

The term "transplantation" refers to any method of administering cells. Thus in one embodiment a surgical procedure the cells are placed in the relevant part of the body. The cells may be administered by direct injection into the relevant site. Preferably the cells are combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition is typically formulated for intravenous or subcutaneous administration, or for administration by transplantation.

In one embodiment the cells are encapsulated. Generally the encapsulating material is permeable to nutrients (such as sugars or amino acids), but impermeable to immune mediators (such as antibodies or complement components) or cells.

Typically the material comprises alginate (alternating blocks of mannuronic and guluronic acid) such as in the form of barium and/or poly-L-lysine alginate. The material may comprise hollow fibres (such as acrylic, polyacrylonitrile vinyl chloride or polyethersulfone). The material may comprise hydroxyethyl-methacrylate-methyl-methacrylate, polyphosphazene or agarose.

The dose of cells which are administered to a patient will depend upon a variety of factors such as the age, weight and general condition of the patient, the disease that is being treated and the particular cells that are being administered. A suitable dose may however be from 0.1 to 100 mg/kg body weight such as 1 to 40 mg/kg body weight, or 10^3 to 10^7 cells. The routes of administration and dosages described above are intended only as a guide since a skilled physician will be able to determine readily the optimum route of administration and dosage for any particular patient and condition.

The inhibitor is administered to a patient by a route which is effective for preventing damage to transplanted cells by fibrils. Suitable routes of administration

and dosages have been discussed above. Generally an effective non-toxic amount of the inhibitor is administered. The inhibitor is typically administered in the form of a pharmaceutical composition comprising the inhibitor in association with a pharmaceutically acceptable carrier or diluent. The inhibitor may be administered in 5 any of the forms discussed above, for example with any of the pharmaceutically acceptable vehicles mentioned above. The inhibitor may be present in any of these forms when it is used in the *in vitro* process of the invention.

The invention is further illustrated by the following examples which should not be construed as further limiting the subject invention. The contents of all 10 references, issued patents, and published patent applications cited throughout this application are hereby incorporated by reference.

Example 1

Determination of the rate of amyloid fibril formation by Thioflavine T spectroscopy

15 Thioflavine T (ThT) binds to amyloid proteins in β -sheet formation, exhibiting a yellow fluorescence from tissue sections and fibrils *in vitro*. Detection of ThT fluorescence can be used as a sensitive assay for amyloid fibril formation under different conditions. This assay has been used in experiments to determine the effects of compounds of the invention on amyloid fibril formation.

Method

Human IAPP was dissolved in 40% trifluoroethanol and freeze-dried into conveniently-sized aliquots. IAPP was prepared immediately before the measurements by dissolving in 40% 1,1,1,3,3-hexafluoro-2-propanol (HFIP) in water to maintain the peptide in alpha helical conformation and soluble. A stock 25 solution of ThT (2.5mM) was prepared, 7.9mg in 10mL Tris-HCl pH 7.0 and filtered (0.22 μ m). Solutions were kept in the dark until use. Fluorescence was examined at 440nm excitation (slit 5nm), and emission at 482nm (slit 10nm) with stirring. 25ml of ThT stock (final concentration 62.5 μ M) was added to peptide sample and made up to 1mL in the cuvette. The sample was stirred for 5 min. before taking a reading. 30 Measurements were made at an initial time point (5 min. from sample preparation), at intervals over the next 4-6h and after overnight incubation at room temperature.

Certain compounds as disclosed herein, i.e., 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid; 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine; cyclohexylsulfamic acid; *O*-phospho-L-serine; hexafluoroglutaric acid; 8-methoxyquinoline-5-sulfonic acid; 3-amino-2-hydroxy-1-propanesulfonic acid; and 3-dimethylamino-1-propanesulfonic acid, and 1,2,3,4-tetrahydroisoquinoline, were found to inhibit or prevent IAPP-associated fibril assembly.

Example 2

10 Circular dichroism analysis was conducted to confirm the activity of certain therapeutic compounds in preventing or inhibiting IAPP-associated fibril formation in accordance with the present disclosure by determining the presence or absence of β -sheet conformation.

The assay is conducted as follows:

15

INSTRUMENT AND PARAMETERS

Instrument: JASCO J-715 Spectropolarimeter

Cell/cuvette: Hellma quartz (QS) with 1.0 mm pathlength

Room temperature

20 Wavelength interval: 250 nm-190 nm

Resolution: 0.1 nm

Band width: 1.0 nm

Response time: 1 sec

Scanning speed: 20 nm/min

25 Number of spectra run: 5

The assay, a co-incubation procedure, examines the ability of a compound or substance to inhibit the assembly of amyloid fibrils, e.g., to test for the presence of the amyloidotic β -sheet conformation in the presence of soluble IAPP. Samples are run in the presence and absence (i.e., water alone) of buffering agent, which is done to determine if competitive effects are seen with the ionic buffer (usually phosphate).

-32-

A. Assay in Water Only

Add components used at a molar ratio of 1:10 [peptide:compound]; add 10 µL of 10 mg/mL IAPP stock solution (final 100 µg peptide) to the aqueous solution containing compound to a final volume of 400 µl. The pH of the final assay solution is measured to ensure there is no fluctuation and the spectrum is accumulated using the parameters as shown above.

5

B. Assay in Phosphate Buffer

Add desired amount of compound to achieve a 1:10 molar ratio in 10 mM phosphate buffer, pH 7. Add 10 µL of 10 mg/mL IAPP stock solution (final peptide 100 µg) to the phosphate buffered solution containing the compound and bring to a final volume of 400 µL. The pH of the final assay solution is measured to ensure there is no fluctuation and the spectrum is accumulated using the parameters as shown above.

10

15

20

In both assays, a control sample is run with each test group. This control contains peptide only in water or buffer at a similar final volume of 400 µl. Spectra for the control are collected initially (first run) and at the end of the test (final run) to ensure that the peptide has not undergone extensive aggregation during the course of the assay. Spectra for the controls are used to compare with the measurements obtained with the treated samples.

CO-INCUBATION:

Make fresh 1 mg/mL stock solution of IAPP in 10 mM phosphate buffer, pH 7. Add desired amount of compound to achieve a 1:10 molar ratio in 10 mM phosphate buffer, pH 7. Incubate for 3 days at room temperature. Make up to final volume of 400 µL with 10 mM phosphate buffer, pH 7. The pH of the final assay solution is measured to ensure there is no fluctuation and the spectrum is accumulated using the parameters as shown above. A similar control is run for comparative purposes.

DATA ANALYSIS

Plots of the spectra (control and treated) are individually assembled and the changes in ellipticity at 218 nm are examined. This minimum is directly correlated with the amount of peptide in β -sheet conformation present in the sample. Changes in either a positive or negative direction are noted and a relative value ("active" or "not active") assigned to the compound as a measure of activity.

	Compound	Activity
10	3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid	Active
	DL-2-amino-5-phosphovaleric acid	Active
	1,2,3,4-tetrahydroisoquinoline, hydrochloride	Active
	cyclohexylsulfamic acid, sodium salt	Active
	O-phospho-L-serine	Active
15	hexafluoroglutaric acid	Active
	8-methoxy-5-quinolinesulfonic acid, sodium salt	Active
	4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine, sodium salt	Active
	3-amino-2-hydroxy-1-propanesulfonic acid	Active
20	3-dimethylamino-1-propanesulfonic acid	Active

Example 3

The synthesis of a compound of the invention, 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine, sodium salt.

To a solution of 4-phenylpyridine (15.5 g, 0.1 mol) in acetone (100 mL) was added 1,3-propane sultone (12.2 g, 0.1 mol) at room temperature. The mixture was then heated at reflux temperature overnight. The resultant suspension was cooled to room temperature. The solid was collected by filtration and washed with acetone.

To a solution of the solid (31 g) in methanol (500 mL) was added sodium borohydride (10 g, 260 mmol) portionwise, and the mixture was stirred at room temperature for 2 h. Distilled water (50 mL) was added to destroy the excess of sodium borohydride. The mixture was diluted with methanol (200 mL), and neutralized with Amberlite IR-120 ion-exchange resin (H^+ form, 300 g). A white precipitate was formed. The precipitate and the resin were removed by filtration and treated with distilled water (400 mL) at ~100 °C. The mixture was filtered and the

residual resin was washed with hot distilled water (2 x 200 mL). The filtrates and washings were combined and concentrated to dryness. The residue was co-evaporated with methanol (3 x 200 mL), and then recrystallized from ethanol-water {8:2 (v/v)} to afford 4-phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine as white 5 crystals (26 g, 93%). The ¹H and ¹³C NMR spectra were in agreement with the structure.

To a solution of 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine (5.6 g, 20 mmol) obtained above in ethanol (180 mL) was added sodium hydroxide (1.2 g, 30 mmol). The suspension was heated at reflux temperature for 30 min. The 10 reaction mixture was then cooled to room temperature. The first crop of product (3.9 g, 64% yield) was collected by filtration. The filtrate was concentrated to dryness, and the residue was recrystallized from ethanol to afford a second crop of product (2 g, 32% yield).

¹H NMR (400 MHz, D₂O): δ 1.85 (quintet, 2 H, J 8.7, 7.7 Hz, 2 H-2'), 2.39- 15 2.45 (m, 4H, 2 H-3' and 2 H-3), 2.59 (t, 2 H, J 5.6 Hz, 2 H-2), 2.80 (t, 2 H, J 7.7 Hz, 2 H-1'), 3.00 (br s, 2 H, 2 H-6), 6.00 (br s, 1H H-5), 7.18-7.36 (m, 5 H, Ar).

¹³C NMR (100.6 MHz D₂O): δ 23.90 (C-2'), 29.01 (C-3), 51.69, 51.76 (C-2, C-3'), 54.45 (C-6), 58.12 (C-1'), 123.75 (C-5), 127.31, 130.01, 131.24 (Ar), 136.89 (C-4), 142.47 (Ar).

20

Example 4

ELISA assay for quantitative determination of fibril formation

Human IAPP (hIAPP) was synthesised by solid state synthesis (Advanced Biotechnologies, UK). Human IAPP forms fibrils immediately in aqueous media. 25 Aliquots of IAPP in water (800μg/ml) were stored frozen at -20°C until use. These samples contain small numbers of fibrils visible with electron microscopy (em).

Two sets of samples containing IAPP (20μM) with or without inhibitors (at a molar ratio of 10:1 with IAPP, (20μM)) were prepared on day 1 in Tris buffer. One set was centrifuged, the supernatant removed and frozen immediately at -20°C (Time zero time point). The other set was incubated overnight at room temp with shaking 30 (time 24h time point). On day 2 these samples were spun and supernatant removed

-35-

to determine the proportion of non-fibrillar IAPP.

To assay IAPP in the supernatant both sets of samples were serially diluted in bicarbonate coating buffer, added to ELISA wells and incubated overnight. Plates were washed in phosphate buffer containing Tween (PBS/Tw) x3. Diluted antiserum raised against human IAPP was incubated on the plate for 90 minutes at 37°C.

Following washing in PBS/Tw, alkaline phosphatase conjugated anti-rabbit antisera was incubated for 90 mins. Following washing in PBS/Tw, alkaline phosphate substrate was added and colour allowed to develop for 30 mins. Optical density was read at 405nm.

Data from all samples was calculated to determine changes of IAPP concentration in the supernatant induced by the candidate substance immediately (i.e. at time zero) or after 24 h incubation. Data in Figure 1 is expressed as the proportion of non-fibrillar IAPP in the supernatant of test samples compared to control at both time points.

Candidate substances (i), (ii), (v), (vi), (ix), (x), the hydrochloride of (iii), and the sodium salt of (iv), (vii) and (viii) were found to increase the proportion of non-fibrillar IAPP in the supernatant. This effect was greater at time zero in samples incubated with (vi) and (x), but greater at time 24 h with all other candidate substances.

Samples of the centrifuged pellet at both time points were examined for changes in morphological characteristics of IAPP fibrils by electron microscopy.

Example 5

Determination of the rate of fibril formation by Thioflavine T spectroscopy

Thioflavine-T binds to proteins in β-sheet formation exhibiting a yellow fluorescence. This can be used as a sensitive assay for fibril formation under different conditions. This assay has been used in experiments to determine the effects of some candidate compounds on fibril formation.

Human IAPP was dissolved in 40% Trifluoroethanol and freeze dried into convenient sized aliquots. IAPP (1mg/ml) was prepared immediately before the measurement by dissolving in 40% HFIP in water to maintain the peptide in alpha

-36-

confirmation and soluble. A stock solution of Thioflavine T (2.5mM) was prepared 7.9mg in 10ml Tris pH 7.0 and filtered (0.22 micron). Solutions were kept in dark until use. Fluorescence was examined at 440nm excitation (slit 5nm), and Emission at 482nm (Slit 10nm) with stirring. Twenty five microlitres of Th-T stock (final conc 62.5 µM) was added to peptide sample (8µg/ml) and made up to 1ml in the cuvette. 5 The sample was stirred for 5 min before taking a reading. Measurements were made at an initial time points (5 mins from sample preparation) and at intervals over the next 4-6h after overnight incubation at room temp with and without candidate compound (vi) and a compound known to accelerate fibril formation, 10 polyvinylsulphonate.

In the thioflavine T assay, polyvinylsulphonate increased the fluorescence units (Figure 2) and compound (vi) reduced fluorescence below the level recorded for IAPP alone. This reduced level was stable for up to 15 hrs. Thus it appears that polyvinylsulphonate increased IAPP fibril formation and (vi) either reduced or 15 prevented fibril formation over this time period.

Example 6

Circular Dichroic Spectroscopy

Human IAPP was prepared in 100% HFIP at 1mg/ml filtered through a 20 0.3µm filter, freeze dried in aliquots, resuspended in 20% HFIP and water, and filtered before analysis. The circular dichroic spectroscopy was performed in the same manner as described in Hubbard *et al* (1991) Biochem J, 275, 785-8. Samples were analysed immediately and after 24 h incubation with candidate substances i, iii, iv and x. Samples of IAPP alone (CNTL) and IAPP with candidate compound 25 showed typical CD spectra for α-conformation at time zero. After 24 h incubation the molecular conformation of IAPP (CNTL) was converted to β-conformation. IAPP in the presence of these candidate compounds remained in α-conformation after 24 h incubation. These data indicate that with time IAPP adopts a β-conformation predicting formation of β-sheet assembly into fibrils. This is prevented by the 30 candidate compounds. The results of the circular dichroic spectroscopy are shown in Figure 3.

Example 7Culture of human islets

Pancreas was obtained from organ donors. Islets isolated by collagenase digestion were either handpicked from the digest or purified on a ficoll gradient.

- 5 Islets were cultured, free floating in bacterial petrie dishes in different media all from Gibco Life Sciences; these included Ham F10, RPMI 1640, CMRL. The media was supplemented with glucose (5.6, 8, 11.1 or 16.7mM), 100u/ml benzpenicillin, 0.1 mg/ml streptomycin and 10% foetal calf serum. The media was changed every two days. Islets were cultured under sterile conditions at 37°C in humidified air/5%
10 Carbon dioxide for periods of 2-10 days. Fibrils formed between cultured cells were identified by electron microscopy and immunogold labelling for IAPP. Figure 4a shows an electron micrograph of isolated human islet cells immunogold labelled for IAPP. The cells were cultured for 6 days in 8mM glucose and RPMI.

15 Culture of mouse islets

These are used as a test system for determining the toxicity and effect of putative amyloid inhibitors in short term culture systems. Whereas normal mice can be used to determine toxicity of compounds added to the media, amyloid fibrils form only in murine islets isolated from transgenic mice expressing the gene for human

- 20 IAPP (transgene incorporation was confirmed by PCR). These islets are isolated in a similar way to that of human islets and are handpicked from the digest and cultured in 16.7mM glucose as above. Amyloid fibrils formed within 4 days of culture and can be quantified by quantitative electron microscopy. Figure 4b shows an electron micrograph of isolated transgenic mouse islet cells which express and secrete
25 human IAPP. The cells were cultured for 6 days in 11.1 mM glucose and RPMI.

Example 9Toxicity Study

- Islets from transgenic mice were cultured in the presence of 50mM and 100mM of the relevant compound and survival was compared with islets cultured in the absence of the compound. Mean islet survival with both concentrations of the
30

-38-

compound is shown in Figure 6 (mean of all experiments with both concentrations + SD). No significant differences were seen between the control (con) and test islets at either concentration with any compound tested.

5 Example 9

Election micrograph analysis of the effect of candidate compounds

Transgenic islets cells were cultured as described above, for 7 days and the media changed every two days. At the end of the culture period the islets were fixed in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.2) for one hour and post-fixed in 1% osmium tetroxide, and embedded in Spurr's resin (Taab Laboratories, Reading, UK) or contrast enhanced in 2% Uranyl Acetate, dehydrated in methanol and embedded in LRGold (Taab, as previous). Ultrathin sections of the islets were mounted on formvar coated nickel grids. Amyloid was identified by immunogold labelling of tissue sections with an antisera raised to human IAPP 1-37, human IAPP 17-37, rat IAPP 1-37 and goat anti-rabbit gold conjugate (10nm diameter) either from Biocell, Cardiff, UK or Sigma or by use of protein A gold. Grids were examined in a JOEL electron microscope at an excitation voltage of 80kV.

Images of 3 islet sections at a low magnification were prepared. A montage of each islet was made from electron micrographs and the areas of both intra and extra cellular amyloid identified and marked. The total islet area, together with the area of intra and extra cellular amyloid was then calculated using image analysis (Kontron Image Processor). 3 separate islets were examined for each condition.

The table below shows total islet area in cultures with and without vii.

25

30

CONTROL	Extra amyloid area (μm^2)	Total islet area (μm^2)	Extra amyloid % of total
islet 1	59.39	3271.48	1.8
islet 2	281.74	4485.74	6.2
islet 3	111.09	3277.28	3.4
Average	150.74	3678.17	4.1
With vii			
islet 1	98.73	5744.23	1.9
islet 2	37.9	7360.63	0.5
islet 3	123.99	3616.76	3.4
Average	86.8	5407.21	1.6

Example 10Morphological characteristics of IAPP fibrils formed in mixtures with candidate compounds.

IAPP prepared as in Example 3 was incubated with candidate compounds for 48 h. Samples were then examined by transmission electron microscopy for the presence of fibrils. A dense network of fibrils was visible in negatively stained preparations of IAPP (1mg/ml) as can be seen in Figure 5a. Long and short unbranching fibrils were present. Samples incubated with compound (ii) contained less fibrils and those present were short and not aggregated (Figure 5b). Samples incubated with compound (vi) also contained fewer fibrils which were apparently short and long and not aggregated (Figure 5c). These data indicate that the process of IAPP fibril formation with time has been reduced by candidate compounds (ii) and (vi).

-40-

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims. The contents of all references, issued patents, and published patent applications cited throughout this application are hereby incorporated by reference.

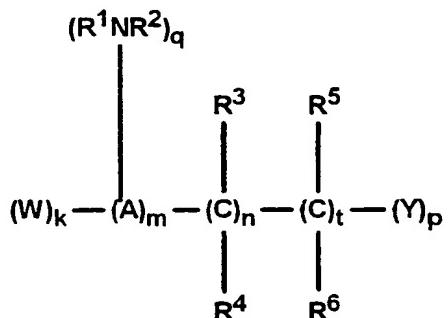
5

-41-

CLAIMS

1. A method for inhibiting IAPP-associated amyloid deposits in a subject, comprising administering to said subject an effective amount of an IAPP fibril inhibiting compound, or a pharmaceutically acceptable salt thereof, such that said IAPP-associated amyloid deposits are inhibited.

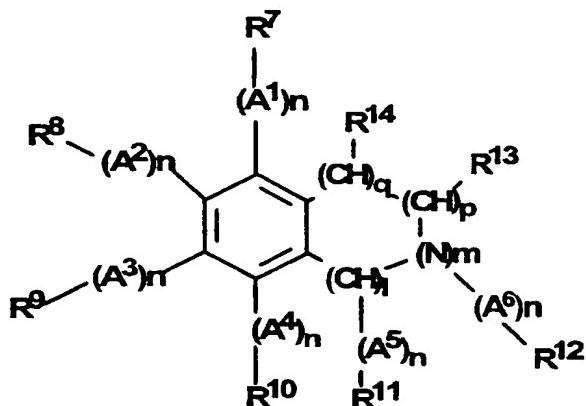
5 2. The method of claim 1 wherein said IAPP fibril inhibiting compound is of the formula



10 15 wherein k, m, t, p and q are independently 0 or 1; n is an integer from 0 to 3; C is a carbon; N is a nitrogen; W is hydrogen or an anionic group at physiological pH; Y is an anionic group at physiological pH; R¹ and R² are independently hydrogen, alkyl, an anionic group at physiological pH, or R¹ and R², taken together with the nitrogen to which they are attached, may form an unsubstituted or substituted heterocycl having from 3 to 7 atoms in the heterocyclic ring; R³ is hydrogen, halogen, thiol or hydroxyl; R⁴, R⁵, and R⁶ are independently hydrogen or halogen; and A is hydrogen or C₁ to C₆ alkyl; or a pharmaceutically acceptable ester, acid or salt thereof.

20 25 3. The method of claim 2, wherein said IAPP fibril inhibiting compound is selected from the group consisting of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid; 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine; cyclohexylsulfamic acid; O-phospho-L-serine; hexafluoroglutaric acid; 8-methoxyquinoline-5-sulfonic acid; 3-amino-2-hydroxy-1-propanesulfonic acid; and 3-dimethylamino-1-propanesulfonic acid, and pharmaceutically acceptable salts thereof.

4. The method of claim 1 wherein said IAPP fibril inhibiting compound is of the formula



wherein C is a carbon; N is a nitrogen; H is a hydrogen; A¹, A², A³, A⁴, A⁵ and A⁶ are independently alkyl, O, S, or -NH; m and n (for each individual A group) are independently 0 or 1; p, q and l are independently 0, 1, or 2; R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², and each R¹⁴ are independently hydrogen, alkyl, alicyclyl, heterocycyl or aryl, each R¹³ is independently hydrogen, alkyl, alicyclyl, heterocycyl, aryl or an anionic group, and adjacent R groups (e.g., R⁷ and R⁸) may form an unsubstituted or substituted cyclic or heterocyclic ring.

5. The method of claim 4 wherein said compound is 1,2,3,4-tetrahydroisoquinoline.

6. The method of claim 1, wherein said IAPP fibril inhibiting compound is administered *in vitro* or *ex vivo*.

25 7. The method of claim 1, wherein said subject has IAPP-associated amyloid deposits in pancreatic islets.

8. A method for inhibiting IAPP fibrillogenesis in a subject, comprising administering to said subject an effective amount of an IAPP fibril inhibiting compound, or a pharmaceutically acceptable salt thereof, such that IAPP fibrillogenesis is inhibited.

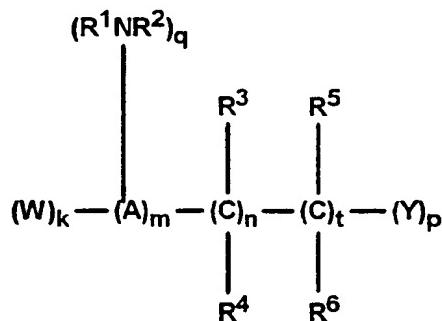
30 9. The method of claim 8, wherein said IAPP fibril inhibiting compound

-43-

is administered *in vitro* or *ex vivo*.

10. A method for reducing IAPP-associated amyloid deposits in a subject having IAPP-associated amyloid deposits, the method comprising administering to said subject an effective amount of an IAPP fibril inhibiting compound, or a
5 pharmaceutically acceptable salt thereof, such that said IAPP-associated amyloid deposits are inhibited.

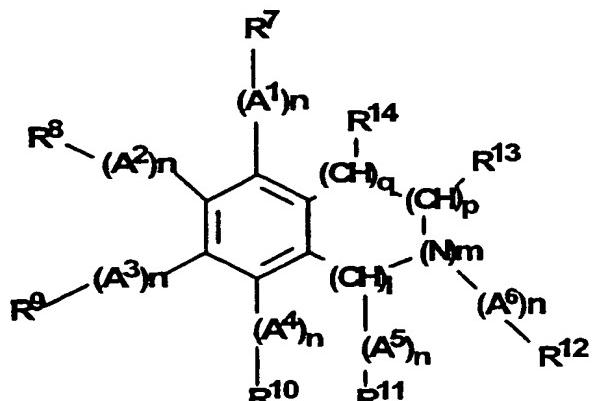
11. The method of claim 10 wherein said IAPP fibril inhibiting compound is of the formula



10 where k, m, t, p and q are independently 0 or 1; n is an integer from 0 to 3; C is a carbon; N is a nitrogen; W is hydrogen or an anionic group at physiological pH; Y is an anionic group at physiological pH; R¹ and R² are independently hydrogen, C₁ to C₄ alkyl, an anionic group at physiological pH, or R¹ and R², taken together with the nitrogen to which they are attached, may form an unsubstituted or substituted heterocycle having from 3 to 7 atoms in the heterocyclic ring; R³ is hydrogen, halogen, thiol or hydroxyl; R⁴, R⁵, and R⁶ are independently hydrogen or halogen; and A is hydrogen or C₁ to C₆ alkyl; or a pharmaceutically acceptable ester, acid or salt thereof.

20
25
30 12. The method of claim 11, wherein said IAPP fibril inhibiting compound is selected from the group consisting of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid; 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine; cyclohexylsulfamic acid; O-phospho-L-serine; hexafluoroglutaric acid; 8-methoxyquinoline-5-sulfonic acid; 3-amino-2-hydroxy-1-propanesulfonic acid; and 3-dimethylamino-1-propanesulfonic acid, and pharmaceutically acceptable salts thereof.

13. The method of claim 10 wherein said IAPP fibril inhibiting compound is of the formula



wherein C is a carbon; N is a nitrogen; H is a hydrogen; A¹, A², A³, A⁴, A⁵ and A⁶ are independently alkyl, O, S, or -NH; m and n (for each individual A group) are independently 0 or 1; p, q and l are independently 0, 1, or 2; R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², and each R¹⁴ are independently hydrogen, alkyl, alicyclyl, heterocycyl or aryl, each R¹³ is independently hydrogen, alkyl, alicyclyl, heterocycyl, aryl or an anionic group, and adjacent R groups (e.g., R⁷ and R⁸) may form an unsubstituted or substituted cyclic or heterocyclic ring.

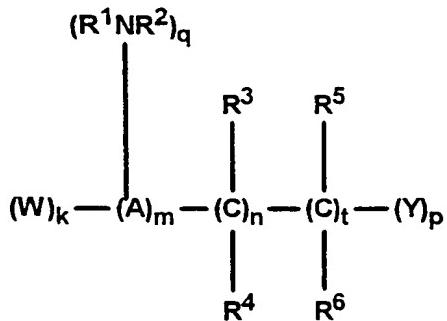
20 14. The method of claim 13 wherein said compound is 1,2,3,4-tetrahydroisoquinoline.

15. The method of claim 10, wherein said IAPP fibril inhibiting compound is administered *in vitro* or *ex vivo*.

25 16. The method of claim 10, wherein said subject has IAPP-associated amyloid deposits in pancreatic islets.

17. A method for inhibiting amyloid deposits in a subject, comprising administering to said subject an effective amount of a compound of the formula

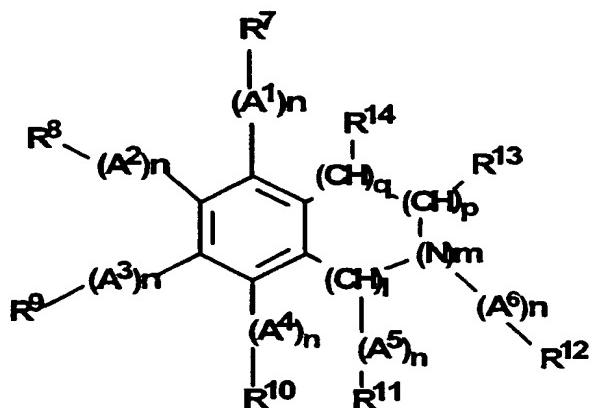
-45-



wherein k, m, t, p and q are independently 0 or 1; n is an integer from 0 to 3; C is a carbon; H is a hydrogen; W is hydrogen or an anionic group at physiological pH; Y is an anionic group at physiological pH; R¹ and R² are independently hydrogen, C₁ to 10 C₄ alkyl, an anionic group at physiological pH, or R¹ and R², taken together with the nitrogen to which they are attached, may form an unsubstituted or substituted heterocycle having from 3 to 7 atoms in the heterocyclic ring; R³ is hydrogen, halogen, thiol or hydroxyl; R⁴, R⁵, and R⁶ are independently hydrogen or halogen; and A is hydrogen or C₁ to C₆ alkyl; or a pharmaceutically acceptable ester, acid or 15 salt thereof, such that said amyloid deposits are inhibited.

18. The method of claim 17, wherein said IAPP fibril inhibiting compound is selected from the group consisting of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid; 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine; cyclohexylsulfamic acid; O-phospho-L-serine; hexafluoroglutaric acid; 8-methoxyquinoline-5-sulfonic acid; 3-amino-2-hydroxy-1-propanesulfonic acid; and 3-dimethylamino-1-propanesulfonic acid, and pharmaceutically acceptable salts thereof.

19. A method for inhibiting amyloid deposits in a subject, comprising administering to said subject an effective amount of a compound of the formula



5

10 wherein C is a carbon; N is a nitrogen; H is a hydrogen; A¹, A², A³, A⁴, A⁵ and A⁶ are independently alkyl, O, S, or -NH; m and n (for each individual A group) are independently 0 or 1; p, q and l are independently 0, 1, or 2; R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², and each R¹⁴ are independently hydrogen, alkyl, alicyclyl, heterocycyl or aryl, each R¹³ is independently hydrogen, alkyl, alicyclyl, heterocycyl, aryl or an anionic group, and adjacent R groups (e.g., R⁷ and R⁸) may form an unsubstituted or substituted cyclic or heterocyclic ring.

15

20. The method of claim 19 wherein said compound is 1,2,3,4-tetrahydroisoquinoline.

21. An IAPP fibril inhibiting compound as defined in any one of claims 1 to 5, or a pharmaceutically acceptable salt thereof, for use in inhibiting IAPP-associated amyloid deposits in a subject.

22. Process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming amyloid deposits, said process comprising contacting the cells *in vitro* with an inhibitor of amyloid deposit formation.

25. Process according to claim 22 wherein said inhibitor causes breakdown of amyloid deposits, the deposits having been formed by said cells prior

to said contacting.

24. Process according to claim 22 or claim 23 in which the cells are cultured in the presence of the inhibitor.

5 25. Process according to any one of claims 22 to 24 wherein the inhibitor is a compound as defined in claim 2.

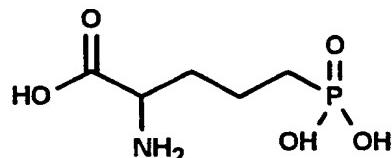
26. Process according to any one of claims 22 to 24 wherein the inhibitor is a compound as defined in claim 4.

27. Process according to any one of claims 22 to 26 wherein the inhibitor is

10 (i) 3-(3-hydroxy-1-propyl) amino-1-propanesulfonic acid

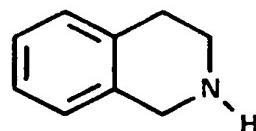


(ii) DL-2-amino-5-phosphovaleric acid



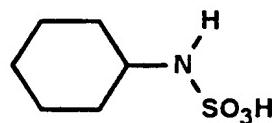
15

(iii) 1, 2, 3, 4 tetrahydroisoquinoline



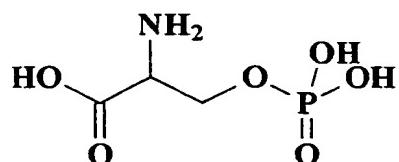
20

(iv) Cyclohexylsulfamic acid

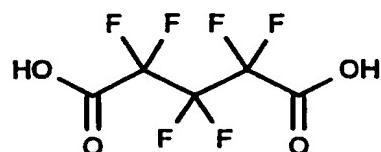


(v) O-Phospho-L-serine

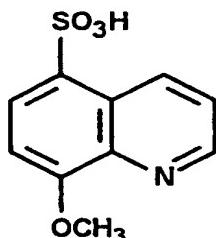
-48-



5 (vi) Hexafluoroglutamic acid

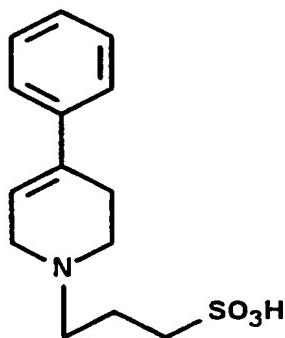


10 (vii) 8-methoxyquinoline-5-sulfonic acid



15

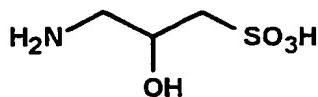
(viii) 4-phenyl-1-(3'-sulfopropyl)- 1, 2, 3, 6 - tetrahydropyridine



20

(ix) 3-amino-2-hydroxy-1- propanesulfonic acid

-49-



or

(x) 3-dimethylamino-1-propanesulfonic acid

5 $\text{Me}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{H}$;

or a salt thereof.

28. Process according to any one of claims 22 to 27 wherein the cells are islet, liver, muscle, kidney, neuronal or stem cells.

10 29. Process according to any one of claims 22 to 28 wherein the cells are human, primate, rodent, rabbit, ovine, porcine, feline or canine cells.

15 30. Process according to any one of claims 22 to 29 wherein the amyloid deposits comprise islet amyloid polypeptide, A β peptide (involved in Alzheimer's disease), prion protein, immunoglobulin light chain, amyloid A protein, transthyretin, cystatin, β 2-microglobulin, apolipoprotein A-1, gelsolin, calcitonin, atrial natriuretic factor, lysozyme variants, insulin, or fibrinogen.

31. Process according to any of claims 22 to 30 wherein the cells are islet cells and the deposits comprise human islet amyloid polypeptide.

32. A culture medium or a culture medium pre-mix which comprises an inhibitor or compound as defined in any one of claims 2, 4, 22 or 27.

20 33. A culture of cells in which the culture medium is as defined in claim 32.

34. A culture according to claim 33 in which the cells are islet cells.

35. *Ex vivo* cells prepared by a process according to any one of claims 22 to 31.

25 36. *Ex vivo* cells according to claim 35 wherein said cells are in a preparation that comprises an inhibitor or compound as defined in any one of claims 2, 4, 27 or 32.

-50-

37. *Ex vivo* cells according to claim 35 or claim 36, wherein the cells are genetically modified.

38. *Ex vivo* cells according to claim 35, 36 or 37 for use in a method of treatment of the human or animal body by therapy.

5 39. *Ex vivo* cells according to claim 38 which are islet cells for use in a method of treating diabetes.

40. *Ex vivo* cells according to claim 38 for use in a method of treating type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, 10 senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen α -chain amyloidosis.

41. A pharmaceutical composition comprising a cell according to claim 15 35, 36 or 37 and a pharmaceutically acceptably carrier or diluent.

42. Use of an inhibitor as defined in any one of claims 2, 4, 22 or 27 in the manufacture of a medicament for inhibiting amyloid deposit formation by, or breaking amyloid deposits down in, a transplanted cell preparation.

43. A vessel for containing a culture of cells, which vessel is coated with 20 an inhibitor or compound as defined in any one of claims 2, 4, 22 or 27.

44. A kit for culturing cells comprising a culture medium or culture medium pre-mix as defined in claim 32 or a vessel as defined in claim 43.

44. Use of an antibody that binds an inhibitor or compound as defined in claim 2, 4 or 27, or of a fragment of said antibody that retains the ability to bind the 25 said inhibitor or compound, to identify a substance that can be used to prepare cells for transplantation in a process according to claim 22 or 23.

46. Method of identifying an inhibitor that can be used to prepare cells for transplantation in a process according to claim 22 or 23, comprising contacting a

-51-

candidate substance with a mammalian cell and determining whether the candidate substance inhibits the formation of fibrils or causes the breakdown of fibrils, (i) the inhibition of formation of fibrils or (ii) the breakdown of fibrils, indicating that the substance is an inhibitor that can be used in said process.

5 47. Method of identifying an inhibitor that can be used to prepare cells for transplantation in a process according to claim 22 or 23, comprising contacting a candidate substance with a protein capable of forming fibrils, or with a fibril, and determining whether the substance inhibits the formation of the protein into a fibril, or whether the substance causes the breakdown of the fibril, (i) inhibition of fibril formation or, (ii) the breakdown of fibrils, indicating that the substance can be used in said process.

10 48. A method according to claim 46 or 47 wherein the mammalian cell is any of the types of cells defined in claim 28, 29 or 31, or the fibrils comprise a protein as defined in claim 30 or 31.

15 49. A inhibitor identified by the use or method of any one of claims 45 to 48.

20 50. A process, culture medium, culture medium pre-mix, culture, *ex vivo* cells, pharmaceutical composition, use, vessel or kit according to any one of claims 22 to 44 wherein the inhibitor is an inhibitor as defined in claim 49.

25 51. Method of inhibiting fibril formation by, or breaking fibrils down in, a transplanted cell preparation comprising administering an inhibitor or compound as defined in any one of claims 2, 4, 22, 27 or 49 to a patient who has received a transplant of cells as defined in claim 35, 36 or 37.

52. Use of a compound as defined in any one of claims 1 to 5 in the manufacture of a medicament for inhibiting IAPP-associated amyloid deposits in a subject.

1/10

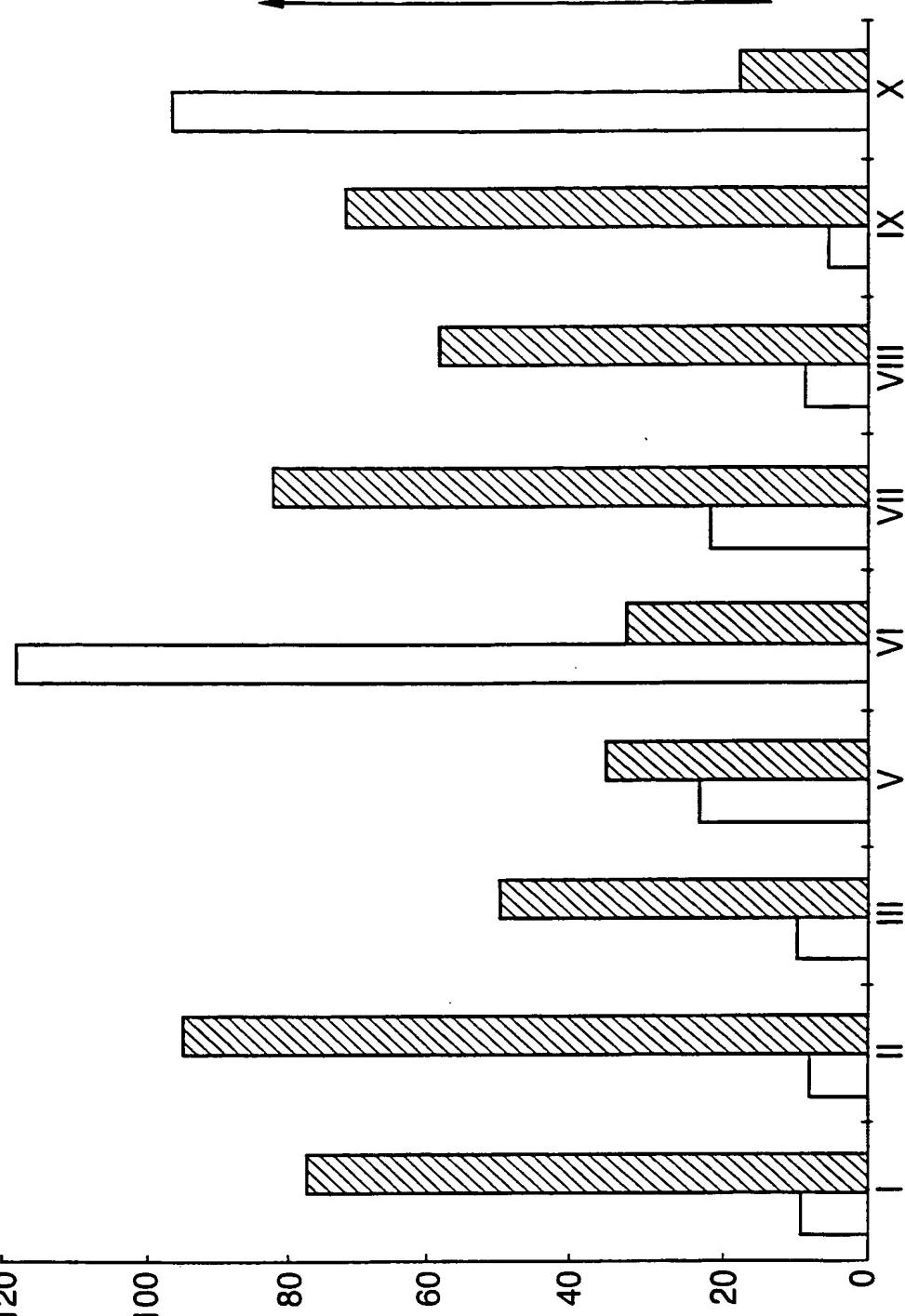
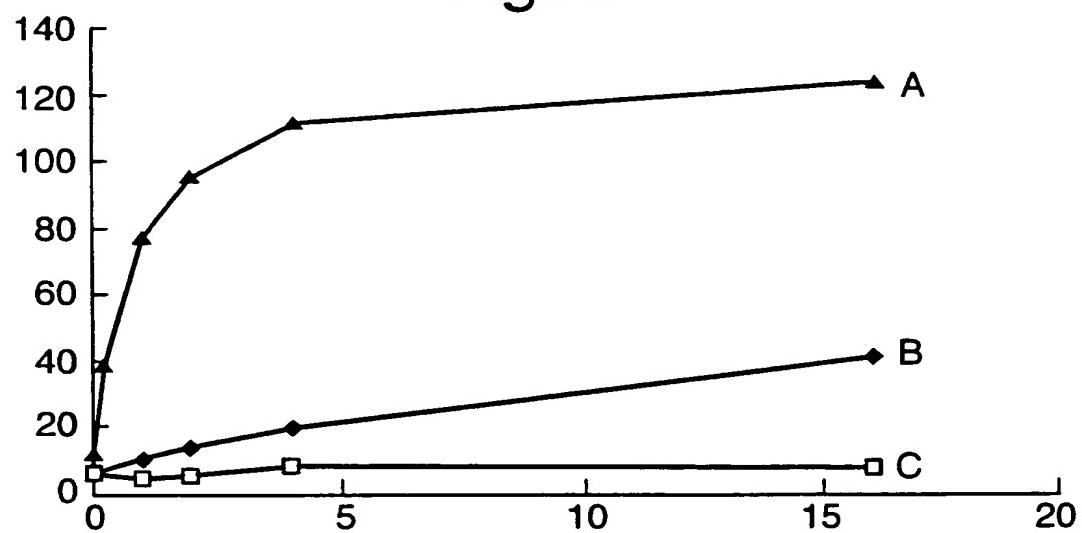


Fig. 1. 120

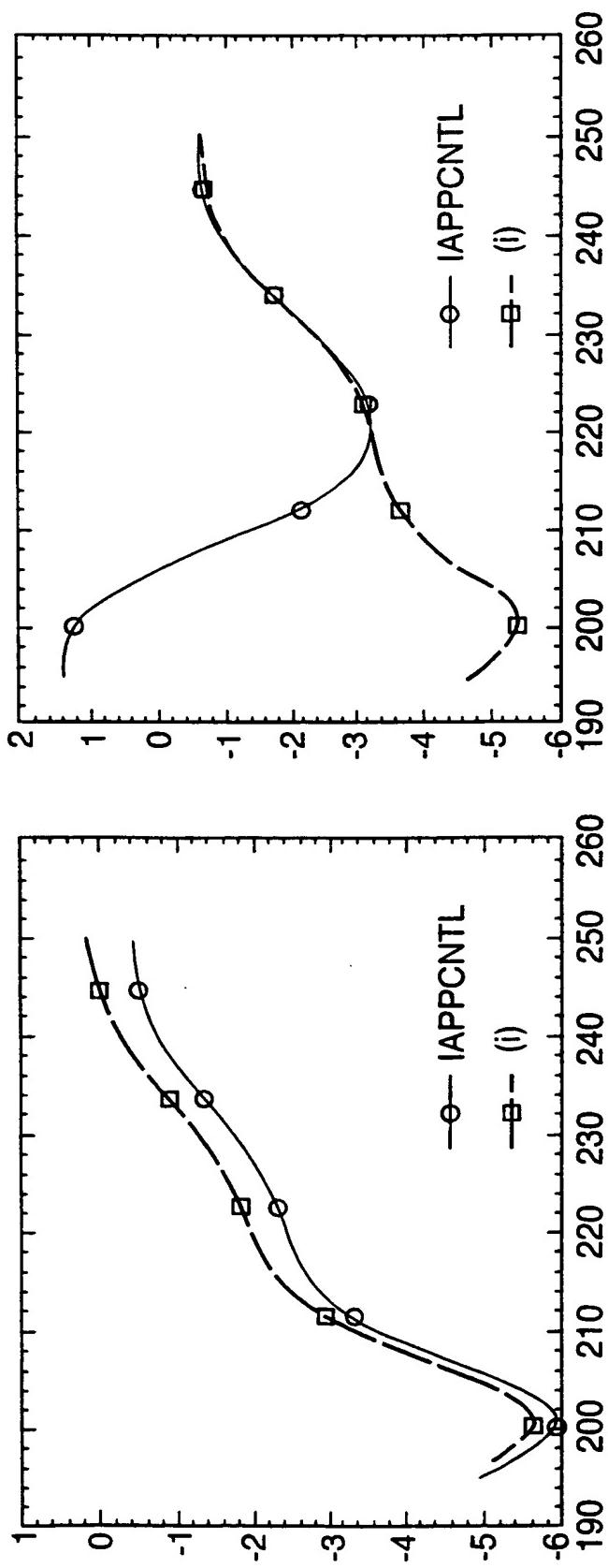
2/10

Fig.2.



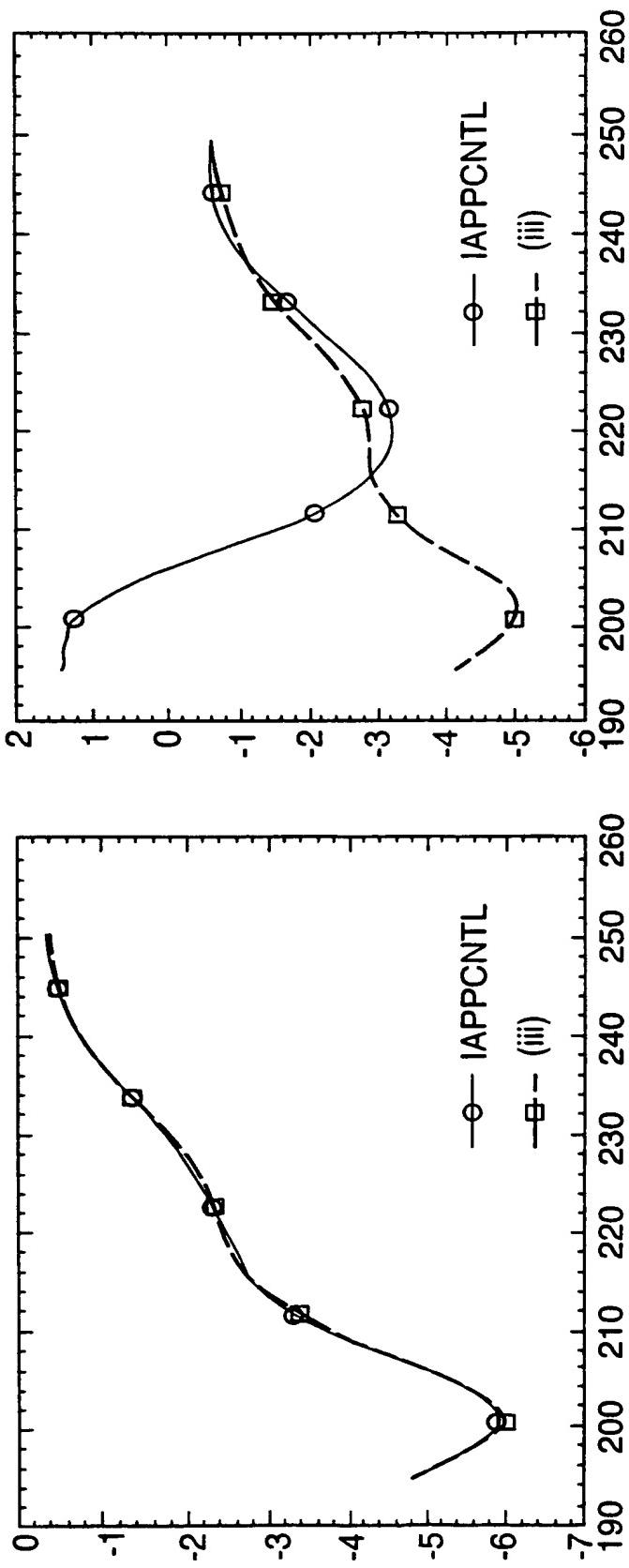
3/10

Fig.3a.



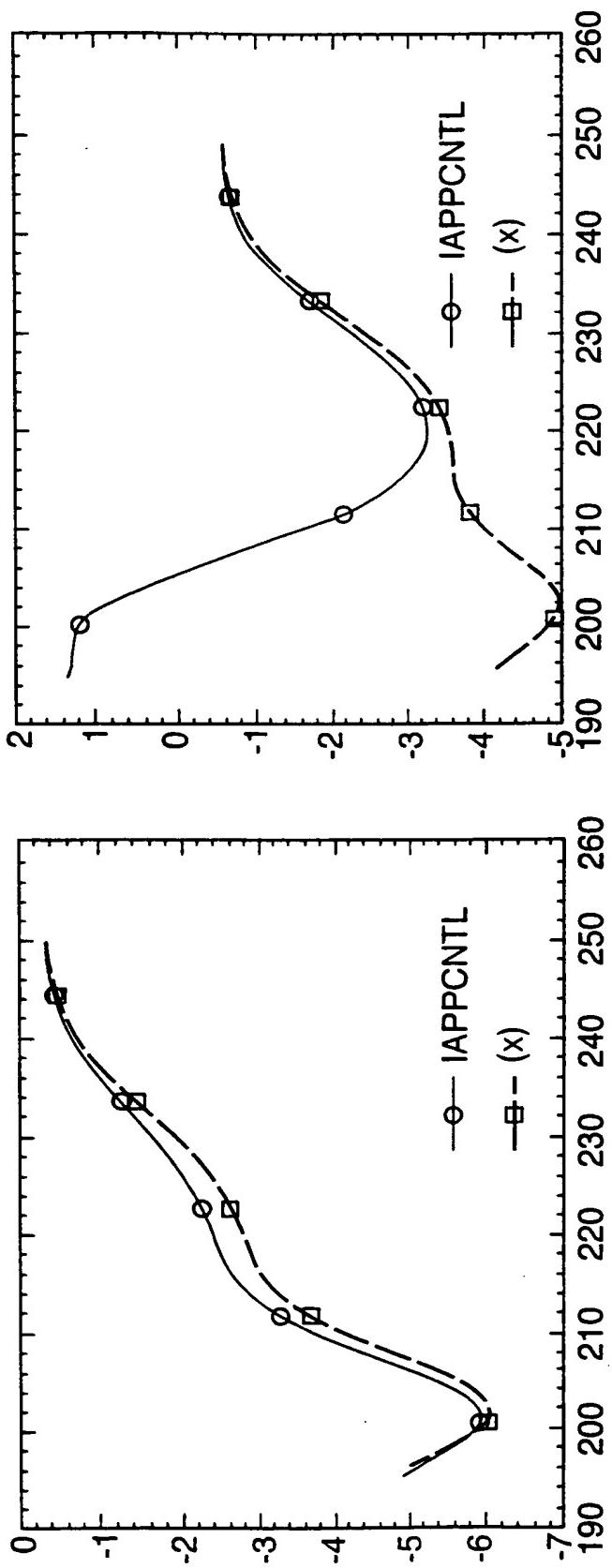
4/10

Fig.3b.



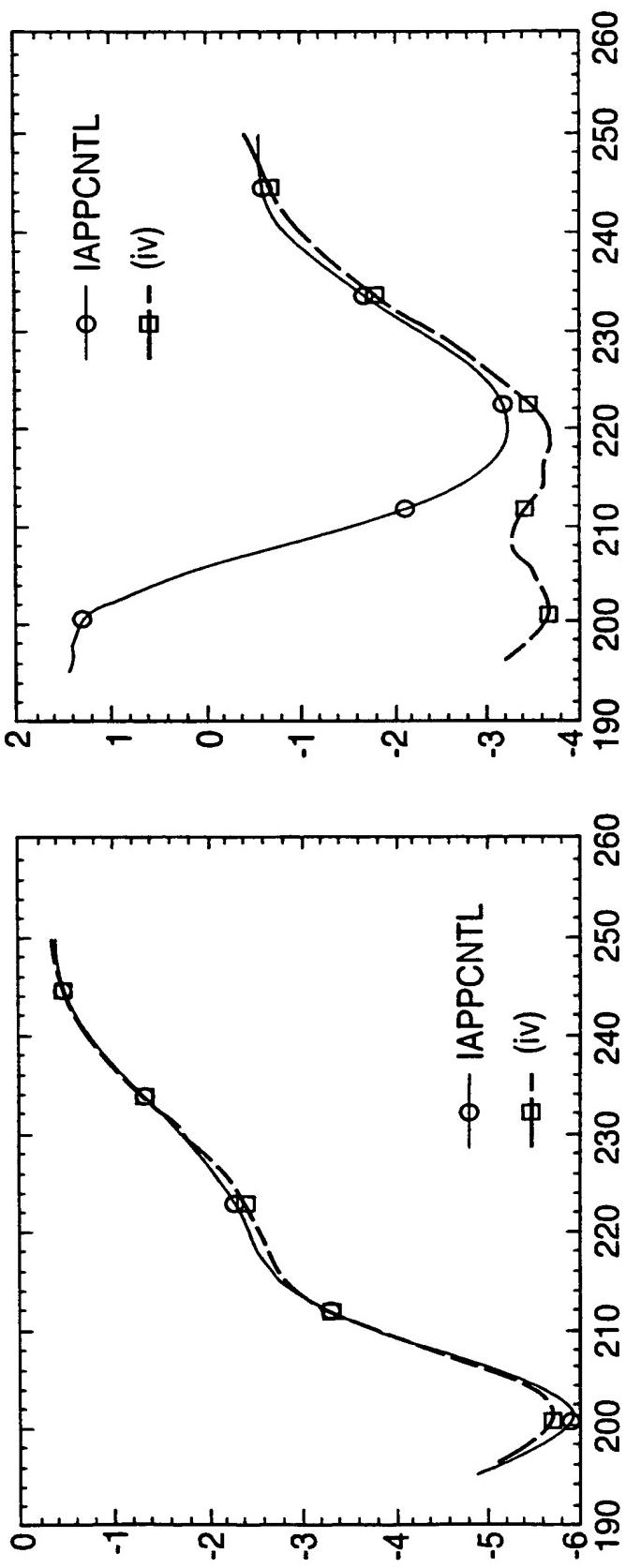
5/10

Fig.3C.



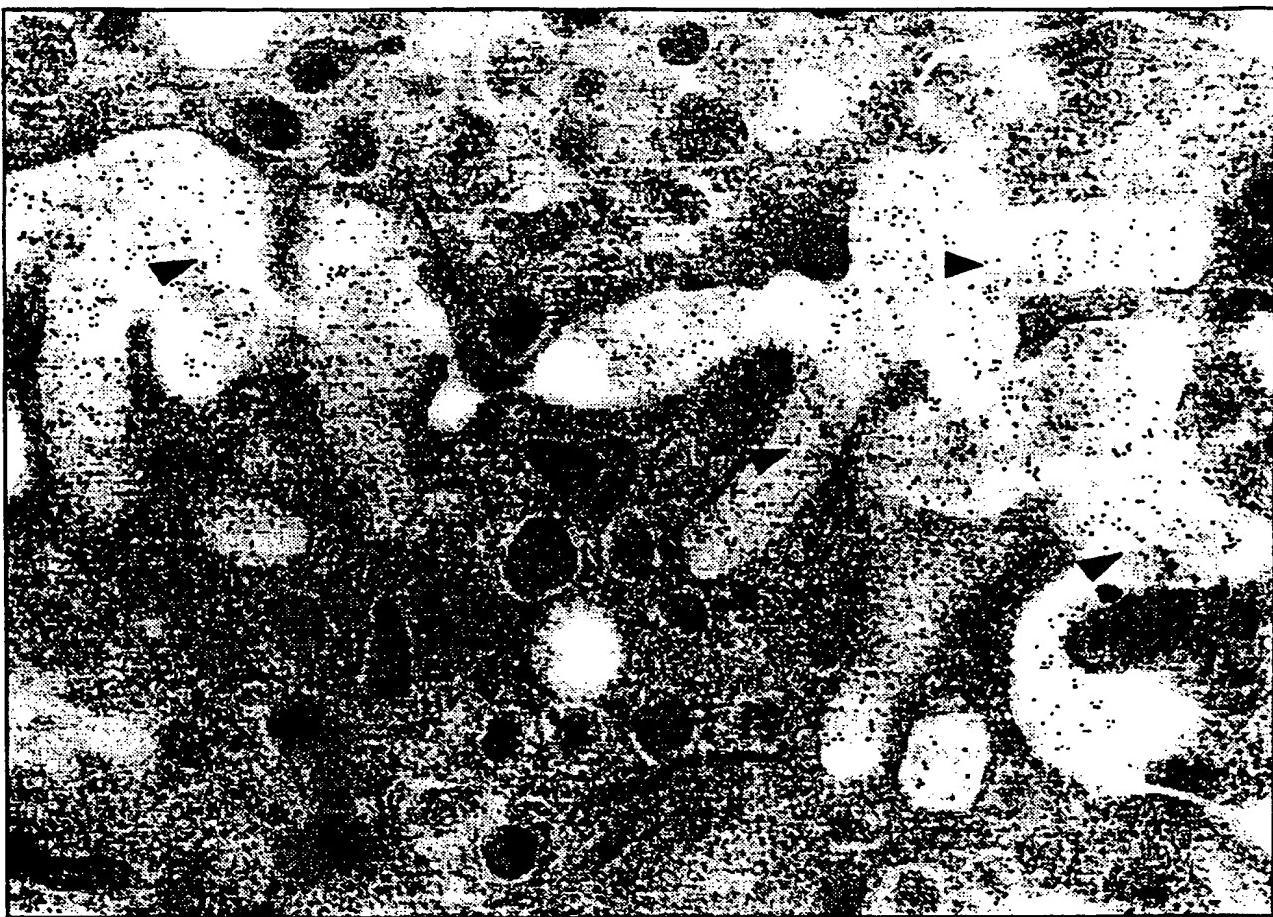
6/10

Fig.3d.



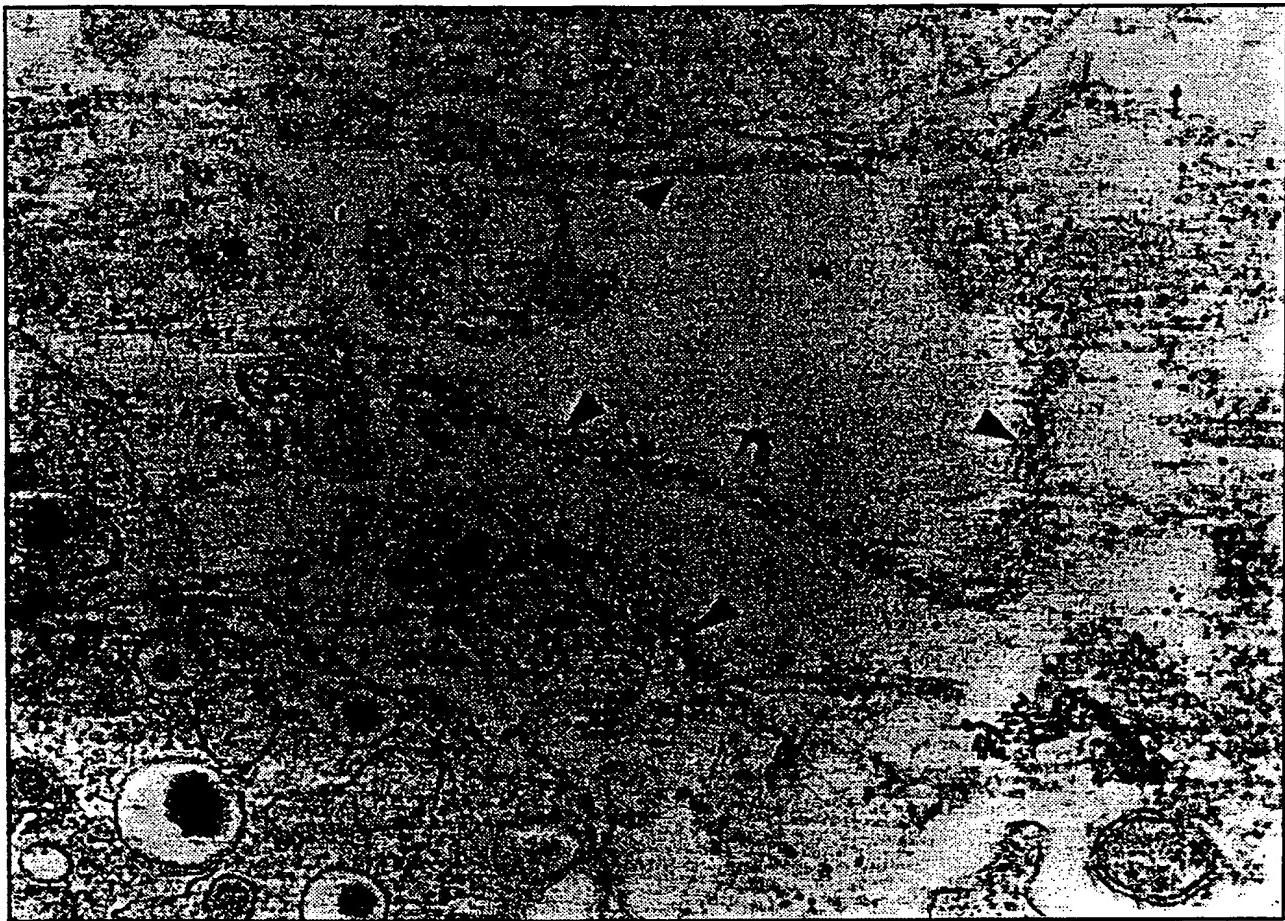
7/10

Fig.4a.



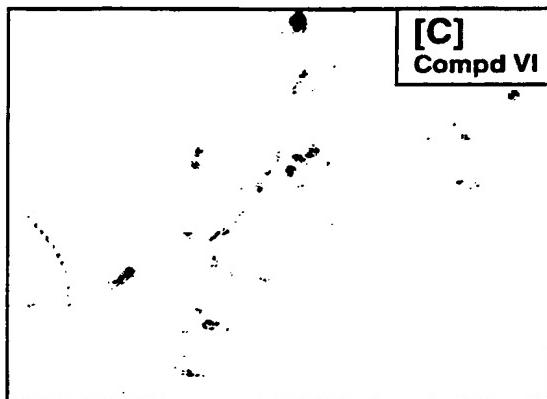
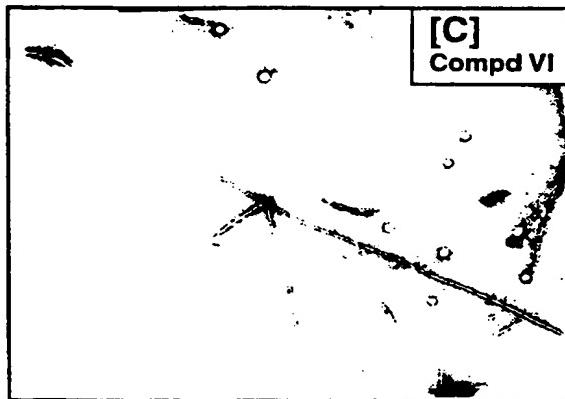
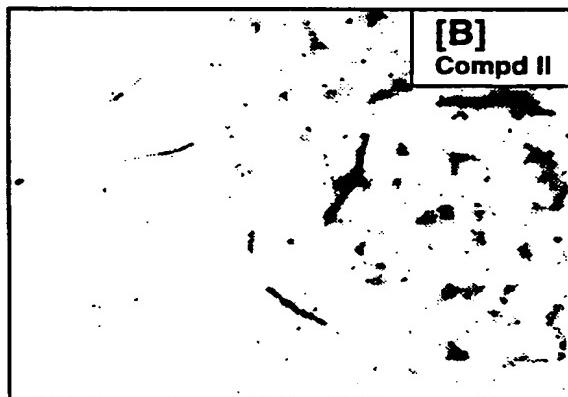
8/10

Fig.4b.

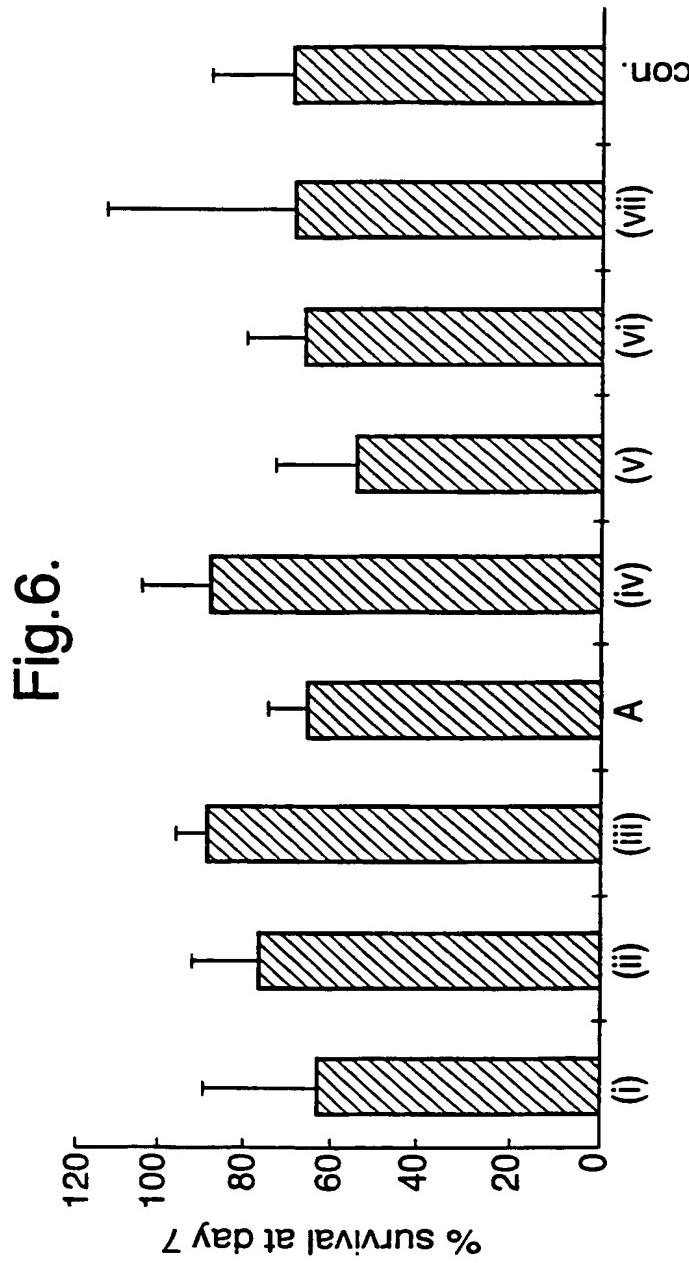


9/10

Fig.5.



10/10



(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 January 2001 (18.01.2001)

PCT

(10) International Publication Number
WO 01/003680 A3

(51) International Patent Classification⁷: A61K 31/145.
31/662, 31/451, 31/445, 31/47, 31/472, A61P 3/00, 25/00

(21) International Application Number: PCT/GB00/02623

(22) International Filing Date: 7 July 2000 (07.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9916214.1 9 July 1999 (09.07.1999) GB
60/142.907 9 July 1999 (09.07.1999) US
9916315.6 12 July 1999 (12.07.1999) GB
60/142.953 12 July 1999 (12.07.1999) US

(71) Applicants (for all designated States except US): ISIS INNOVATION LIMITED [GB/GB]; Ewert House, Ewert Place, Summertown, Oxford OX2 7BZ (GB). NEUROCHEM, INC. [CA/CA]; 7220 Frederick-Banting, Suite 100, Montreal, Quebec H4S 2A1 (CA). QUEENS UNIVERSITY AT KINGSTON [CA/CA]; Kingston, Ontario K7L 3N6 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): CLARK, Anne [GB/GB]; Diabetes Research Laboratories, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE (GB). FRASER, Paul [CA/CA]; Centre for Research into Neurodegenerative Diseases, University of Toronto, 6 Queens Park Crescent West, Toronto M5S 3H2 (CA). VERCHERE, Brigitte [CA/CA]; Department of Pathology and Laboratory Medicine, British Columbia Research Institute for Children and Women's Health, 950 West 28

Avenue, Vancouver, British Columbia (CA). GUPTA, Ajay [CA/CA]; 255 St-Louis, Pointe-Claire, Québec H9R 5L6 (CA). MIGNEAULT, David [CA/CA]; 1125 Haendel, Laval, Québec H7W 3A1 (CA). SZAREK, Walter [CA/CA]; 165 Ontario Street, Suite 301, Kingston, Ontario K7L 2Y6 (CA). WEAVER, Donald [CA/CA]; 27 College View Crescent, Kingston, Ontario K7M 7J8 (CA).

(74) Agents: IRVINE, Claire, Jonquil et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:
11 July 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/003680 A3

(54) Title: COMPOUNDS FOR INHIBITING DISEASES AND PREPARING CELLS FOR TRANSPLANTATION

(57) Abstract: Methods and compositions are provided for inhibiting, preventing and treating amyloid depositions, e.g. in pancreatic islets, wherein the amyloidotic deposits are islet amyloid polypeptide (IAPP)-associated amyloid deposition or deposits. Accordingly, the compositions and method of the invention are useful for inhibiting diabetes. The invention also provides a process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming fibrils, said process comprising contacting the cells with an inhibitor of fibril formation. In particular the process prepares cells for use in a method of treating diabetes. Also provided are a culture medium comprising the inhibitor more particularly selected from the group consisting of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid; DL-2-amino-5-phosphovaleric acid; 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine, cyclohexylsulfamic acid, O-phospho-L-serine, hexafluoroglutaric acid, 8-methoxyquinoline-5-sulfonic acid, 3-amino-2-hydroxy-1-propanesulfonic acid, and 3-dimethylamino-1-propanesulfonic acid and 1,2,3,4-tetrahydroisoquinoline.

INT'L. NATIONAL SEARCH REPORT

International Application No

PCT/GB 00/02623

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 7	A61K31/145	A61K31/662	A61K31/451	A61K31/445	A61K31/47
	A61K31/472	A61P3/00	A61P25/00		

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBASE, BIOSIS, CHEM ABS Data, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 37612 A (PFIZER ;CARTY MAYNARD D (US); KREUTTER DAVID K (US); SOELLER WALTE) 28 November 1996 (1996-11-28) abstract page 2, line 16 -page 4, line 3 ---	1
X	WO 94 22437 A (UNIV KINGSTON) 13 October 1994 (1994-10-13) abstract page 1, line 33 -page 2, line 36 page 4, line 16-32 page 13, line 10-17 page 15, line 21 -page 16, line 2; claim 1 --- -/-	1,2,8, 10,11, 17,21,52

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

10 December 2001

Date of mailing of the international search report

27.03.2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

A. Jakobs

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/02623

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 28187 A (KISILEVSKY ROBERT ;SZAREK WALTER (CA); UNIV KINGSTON (CA); WEAVER) 19 September 1996 (1996-09-19) abstract page 5, line 1 -page 6, line 5 page 12, paragraph 4 -page 14, paragraph 1 page 17, paragraph 1; table 2 ---	1,2,8, 10,11, 17,21,52
X	WO 94 27602 A (CORTEX PHARMA INC) 8 December 1994 (1994-12-08) abstract page 3, line 16 -page 4, line 15 page 4, line 21 -page 6, line 31 page 9, line 23 -page 10, line 25; claim 16; example 16 ---	1,2
X	"USE OF METABOTROPIC AGONISTS IN PROGRESSIVE NEURODEGENERATIVE DISEASES" EXPERT OPINION ON THERAPEUTIC PATENTS, ASHLEY PUBLICATIONS, GB, vol. 5, no. 5, 1995, pages 491-493, XP001012270 ISSN: 1354-3776 the whole document ---	1,2
X	SCHWARCZ R. ET AL: "Excitatory aminoacid antagonists provide a therapeutic approach to neurological disorders." LANCET, (1985) 2/8447 (140-143). CODEN: LANCAO, XP001030857 abstract page 141, column 2, paragraph 3 -page 143, column 1, paragraph 2 ---	1,2
X	WO 93 03714 A (UPJOHN CO) 4 March 1993 (1993-03-04) abstract page 4, line 33 -page 6, line 5 page 11, line 1-25; claims 1-9; tables 3,6,8 ----	1,2,7,8, 10,11, 16,17, 21,52

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/02623

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COPANI A ET AL: "ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS PROTECTS CULTURED NEURONS AGAINST APOPTOSIS INDUCED BY beta-AMYLOID PEPTIDE" MOLECULAR PHARMACOLOGY, BALTIMORE, MD, US, vol. 5, no. 47, 1995, pages 890-897, XP002079923 ISSN: 0026-895X abstract page 890, column 1-2; figure 3 page 893, column 2, paragraph 2 -page 894, column 1, paragraph 1; figure 5 page 895, column 2, paragraph 1 -page 896, column 1, paragraph 2 ---	1,2
X	HUTCHINGS R ET AL: "The effect of excitotoxin antagonists on ibotenic acid-induced alteration of APP mRNA hippocampal expression." JOURNAL OF PHARMACY AND PHARMACOLOGY, vol. 47, no. 12B, 1995, page 1131 XP002079922 British Pharmaceutical Conference 1995: Science Proceedings of the 132nd Meeting; Warwick, England, UK; September 15-18, 1995 ISSN: 0022-3573 abstract	1,2
X	PACHE D M ET AL: "EFFECT OF SELECTIVE EXCITATORY AMINO ACID ANTAGONISTS ON EXCITOTOXIN-INDUCED CHANGES IN APP MRNA EXPRESSION" PHARMACOLOGY REVIEWS AND COMMUNICATIONS, GORDON AND BREACH, CH, vol. 10, no. 3, 1999, pages 205-212, XP001014486 abstract	1,2
X	WO 98 11923 A (Baylor College Medicine ;GIULIAN DANA J (US)) 26 March 1998 (1998-03-26) the whole document	1
E	WO 00 71101 A (NEUROCHEM INC ;UNIV KINGSTON (CA)) 30 November 2000 (2000-11-30) the whole document	1-3, 7, 8, 10-12, 16-18, 21, 52
		-/-

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/02623

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 64420 A (KONG XIANQI ;NEUROCHEM INC (CA); SZAREK WALTER (CA); UNIV KINGSTON) 2 November 2000 (2000-11-02) abstract page 2, line 15 -page 4, line 25 page 13, line 10 -page 15, line 13 page 22, line 21 -page 23, line 12; claims 1-3,9,12-16,20-22,25-65; examples 3-8 ---	1-3,7,8, 10-12, 16-18, 21,52
X,P	WO 99 40909 A (NEUROCHEM INC) 19 August 1999 (1999-08-19) abstract page 3, line 3-14 page 12, line 7-19 claims 1-12 -----	1-3,7,8, 10-12, 16-18, 21,52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/02623

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-3,7,8,10-12,16-18,21,52 (partially)

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 3-(3-hydroxy-1-propyl)amino-1-propanesulfonic acid, 3-amino-2-hydroxy-1-propanesulfonic acid, and 3-dimethylamino-1-propanesulfonic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

2. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of DL-2-amino-5-phosphovaleric acid for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

3. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 4-Phenyl-1-(3'-sulfopropyl)-1,2,3,6-tetrahydropyridine for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

4. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of cyclohexylsulfamic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

5. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of O-phospho-L-serine for inhibiting IAPP-associated amyloid deposits in a disorder where such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

6. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of hexafluoroglutaric acid for inhibiting IAPP-associated amyloid deposits in a disorder where such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

7. Claims: 1-3,7,8,10-12,16-18,21,52 (partially)

Use of 8-methoxyquinoline-5-sulfonic acid for inhibiting IAPP-associated amyloid deposits in a disorder where such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. Claims: 1,8,10,16,21 (partially) 4,5,13,14,19,20

Use of compounds of formula of claim 4, including 1,2,3,4-tetrahydroisoquinoline for inhibiting IAPP-associated amyloid deposits in a disorder where such such amyloid deposition occurs, such as type I or type II diabetes, Alzheimer's disease, a spongiform encephalopathy, primary or secondary systemic amyloidosis, familial amyloidotic polyneuropathy, senile systemic amyloidosis, hereditary cerebral amyloid angiopathy, haemodialysis-related amyloidosis, Finnish hereditary amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, lysozyme amyloidosis, or fibrinogen a-chain amyloidosis.

9. Claims: 6,9,15,22-44(1)

Process for the preparation of a cell according to claims 22-31, ex vivo cells produced by such process according to claims 35-41, and culture mediums according to claim 32-34, a vessel and kit according to claims 43-44.

10. Claims: 44(2),46-51

Use of an antibody that binds an inhibitor or compound as defined in claim 2,4 or 27, or of a fragment of said antibody that retains the ability to bind the said inhibitor or compound, to identify a substance that can be used to prepare cells for transplantation in a process according to claim 22 or 23 and a method of identifying an inhibitor that can be used to prepare cells for transplantation in a process according to claim 22 or 23, comprising contacting a candidate substance with a mammalian cell and determining whether the candidate substance inhibits the formation of fibrils or causes the breakdown of fibrils, (i) the inhibition of formation of fibrils or (ii) the breakdown of fibrils, indicating that the substance is an inhibitor that can be used in said process, and inhibitors identified by such method (sic).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-21,52. relate to a product/therapeutic application defined by reference to a desirable characteristic or property, namely inhibiting IAPP-associated amyloid deposits.

The claims cover all product/therapeutic application having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such product/therapeutic application. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/therapeutic application by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Present claims 2,11 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the product/therapeutic application relating to the treatment of diabetes (see claim 7) as far as related to the first invention.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/02623

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 9637612	A	28-11-1996	US	6187991 B1		13-02-2001
			CA	2219629 A1		28-11-1996
			EP	0827540 A1		11-03-1998
			WO	9637612 A1		28-11-1996
			JP	3258024 B2		18-02-2002
			JP	10507084 T		14-07-1998

WO 9422437	A	13-10-1994	CA	2159326 A1		13-10-1994
			CA	2159649 A1		13-10-1994
			WO	9422437 A2		13-10-1994
			WO	9422885 A1		13-10-1994
			EP	1060750 A2		20-12-2000
			EP	0691844 A1		17-01-1996
			EP	0691976 A1		17-01-1996
			JP	8508260 T		03-09-1996
			US	5643562 A		01-07-1997
			US	5972328 A		26-10-1999
			US	5728375 A		17-03-1998
			US	5840294 A		24-11-1998
			US	2001048941 A1		06-12-2001

WO 9628187	A	19-09-1996	US	5643562 A		01-07-1997
			US	5972328 A		26-10-1999
			US	5840294 A		24-11-1998
			AU	716218 B2		24-02-2000
			AU	5097696 A		02-10-1996
			BR	9607197 A		11-11-1997
			CA	2213759 A1		19-09-1996
			WO	9628187 A1		19-09-1996
			EP	0814842 A1		07-01-1998
			JP	11501635 T		09-02-1999
			US	5728375 A		17-03-1998
			US	2001048941 A1		06-12-2001

WO 9427602	A	08-12-1994	AU	6836394 A		20-12-1994
			WO	9427602 A1		08-12-1994
			US	5622981 A		22-04-1997

WO 9303714	A	04-03-1993	AU	664710 B2		30-11-1995
			AU	2407592 A		16-03-1993
			AU	3061495 A		09-11-1995
			AU	3061595 A		09-11-1995
			CA	2113817 A1		04-02-1993
			EP	0600973 A1		15-06-1994
			JP	6510760 T		01-12-1994
			WO	9303714 A2		04-03-1993

WO 9811923	A	26-03-1998	US	6071493 A		06-06-2000
			US	6043283 A		28-03-2000
			AU	738509 B2		20-09-2001
			AU	4589497 A		14-04-1998
			EP	1051195 A1		15-11-2000
			WO	9811923 A1		26-03-1998
			US	2001016326 A1		23-08-2001
			US	2001016327 A1		23-08-2001

WO 0071101	A	30-11-2000	AU	4282400 A		10-11-2000
			AU	4905000 A		12-12-2000

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/02623

Patent document cited in search report	Publication date		Patent family member(s)		Publication date
WO 0071101	A		WO 0064420 A2		02-11-2000
			WO 0071101 A2		30-11-2000

WO 0064420	A	02-11-2000	AU 4282400 A		10-11-2000
			WO 0064420 A2		02-11-2000
			AU 4905000 A		12-12-2000
			WO 0071101 A2		30-11-2000

WO 9940909	A	19-08-1999	AU 2437899 A		30-08-1999
			CA 2320224 A1		19-08-1999
			EP 1054664 A1		29-11-2000
			WO 9940909 A1		19-08-1999
			JP 2002502871 T		29-01-2002
			US 2002022657 A1		21-02-2002
